

INTERFACIAL FORCES
AND PHENOMENA IN
PHYSIOLOGY

SIR WILLIAM M. BAYLISS

PHYSIOLOGICAL LABORATORY,
UNIVERSITY OF CAMBRIDGE

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**INTERFACIAL FORCES AND
PHENOMENA IN PHYSIOLOGY**

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INTERFACIAL FORCES AND PHENOMENA IN PHYSIOLOGY

BEING THE HERTER LECTURES IN
NEW YORK IN MARCH, 1922

BY

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PREFACE

THE following pages consist of a report, in somewhat expanded form, of the Lectures given at New York in March, 1922, under the auspices of the Herter Foundation. Although a large number of the facts to which reference is made are more or less well known to physiologists it is thought that a good purpose may be served by bringing them into relationship with one another by means of certain elementary principles, which are apt to be forgotten. There is moreover some risk that significant facts may be overlooked on account of their publication in places not readily accessible, or on account of their being subsidiary to the main argument of a

particular paper. An attempt is made to bring some of these facts into place as contributions to a general theory.

It has been found impossible to avoid criticism of some rather widely current views, as also to neglect the indication of gaps where our knowledge is not so satisfactory as is sometimes imagined. When such criticism is made, it is far from my intention to undervalue the work done by those who have put forward what I am bound to look upon as incorrect interpretations. My object is rather to warn against the danger, which is by no means absent, of supposing that everything is clear in these particular instances and also, as I hope, to suggest problems for further work.

The remark might naturally be made by a reader of the list of contents that the greater part of physiology appears to be included. To avoid misconception, it may

be stated here that it is only certain aspects of most of the phenomena mentioned that receive more than a brief reference. The aspects in question are, of course, those in which the properties of surfaces come into especial prominence.

If it be thought that undue emphasis is sometimes laid on the author's own work, it may be called to mind that one of the objects of the Lectures is to enable workers to give an account of their own researches.

W. M. BAYLISS

INTERFACIAL FORCES AND PHENOMENA IN PHYSIOLOGY

CHAPTER I

HETEROGENEOUS SYSTEMS

THE subject with which the following pages is concerned, is that of the phenomena which make themselves manifest at the place or surface where two substances that do not mix together are in contact. Since each of these substances has its own surface, which touches that of the other, it is usual to refer to the common surface as the "interface." This interface may be looked upon as composed, in a sense, of both substances. Although our proper subject is the way in which such phenomena play a part in various physiological processes, it will be advisable to

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devote some preliminary discussion to the phenomena in general.

These systems composed of substances which, although in contact and so far having an influence on one another, are separate and distinct from each other, are known as "heterogeneous systems." It will be clear that if we imagine ourselves moving about in such a system we should find ourselves in different surroundings at different times, whereas in a solution of salt in water we should be always in situations alike to one another. In other words, if we take samples from different parts of a heterogeneous system, they will not necessarily be of the same composition, whereas they will be so if taken from a homogeneous system.

It needs no apology for bringing forward in the country of Willard Gibbs a plea for more investigation of heterogeneous systems, since it is to him that we owe the first systematic investigation of the conditions which control their behaviour. A large amount of attention is being given to

the chemical reactions in homogeneous systems, such as those occurring in true solutions, and rightly so. But, although the investigation of heterogeneous systems is growing in interest and importance, this aspect is not sufficiently considered as it applies to the phenomena of living organisms. It is scarcely necessary to point out that the cells of these beings are heterogeneous systems. They consist of various kinds of structures which are distinct from one another in space, and it is in contact with and under the influence of the surfaces of these structures that the chemical changes take place. This is not intended to imply in any way that there are no important homogeneous reactions in the living organism, but to point out that where heterogeneity exists incorrect conclusions must result if it is neglected.

The molecules which make up the outer surface of any mass of matter are clearly exposed to influences which differ from those in the interior. Thus, on that side turned towards the interior, the surface

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molecules are in similar circumstances to those inside the mass; the forces acting on them are exerted by molecules like themselves. Whereas on the outer side they are exposed to the influence of molecules of nature unlike themselves. We naturally expect to find, therefore, that the phenomena present at the surface will be in some respects unlike those of the interior.

Consider, for example, how the law of mass action is to be applied. It will be remembered that this law tells us that the rate of a reaction is proportional to the concentration of the substances reacting, but that the concentration which is meant is that part of the substance which is actually "active" and is only, even in homogeneous systems, occasionally identical with the total mass present. If it is the hydrogen ions of an acid that react, we have to take into account the degree of dissociation of the acid. Or if we accept the view that strong electrolytes are always completely dissociated, although not all

the ions are active, we must introduce a correction for the "activity." In such cases, however, it is usually comparatively easy to determine the "activity constant." In the case of a heterogeneous reaction, on the contrary, the matter is not so simple. It is obvious that the only molecules accessible to other foreign molecules are those on the surface of the masses of dimensions varying in wide degree. Hence the active mass is proportional to the surface and the difficulty comes in when we try to measure this surface. Hence the direct application of mass action formulæ is inadmissible. This does not mean that mass action is not always a controlling factor. The difficulty lies in the finding out what actually is the mass to be reckoned with.

It is nevertheless remarkable that reactions in heterogeneous systems are sometimes, at all events in part of their course, found to obey fairly well the ordinary unimolecular law, in which the rate at any moment is proportional to the concentration of the reagents which have

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not yet entered into activity. As we know that some of these cases are complex series of reactions, the danger of interpreting them as simple chemical reactions because the whole process appears to follow the unimolecular law should be obvious. In those cases where a series of different kinds of processes occurs, the controlling factor is the slowest one, and it may be that this is a simple chemical reaction. The fact that a particular phenomenon obeys the unimolecular law proves very little. Many processes which are not chemical progress at a rate which is proportional to what is left unchanged, or the amount present at any moment. Money at compound interest obeys such a law, which indeed is sometimes called the "compound interest law."

The danger of drawing conclusions from the fact that a process follows a simple mathematical expression is well shown in the case of the increase in rate of the beat of the isolated mammalian heart with rising temperature. It has been found that this rate is directly proportional to

the absolute temperature, just as simple physical phenomenon such as the expansion of a gas. The conclusion might be drawn that no chemical process occurs in the heart and that the beat is a purely physical process, which is absurd.

We shall meet with many cases in which we cannot limit our interpretations to either chemical or physical laws alone. It would be safe to say that there is scarcely any physiological process in which both physical and chemical factors do not take part. For this reason, the separation of physiology into bio-physics and bio-chemistry seems to me to be undesirable, however convenient it may be sometimes for practical purposes. The theoretical treatment cannot logically be so divided and the laboratories ought always to be in close co-operation.

When we come to discuss phenomena in which the behaviour of atoms and molecules is concerned, we shall see how difficult or impossible it is to distinguish between chemistry and physics. They become one

in this region, and the time is approaching when we shall demand an explanation of chemical facts in terms of the physics of the atom—loss or gain of electrons, change of position of electrons, taking on of electrons in common and so on.

Different substances appear to unite together in ways which show all degrees of transition between physical and chemical modes. At one extreme, we have compounds like the metallic oxides, water, or the enormous variety of organic compounds, where the properties are completely changed. At the other, the taking up of water by silica, where the process is continuous in relation to the vapour pressure of the water, or of gases by charcoal and so on. Between these, there is the case of water of crystallization or of hydration, where definite molecular proportion is present, although there is little if any change in chemical properties.

Some years ago, Noyes directed attention to two different kinds of chemical affinity. Such a compound as hydrochloric acid,

for example, arises from the union of a positive and a negative ion with mutual neutralization of electric charge, in which the excess electron possessed by the negative ion is handed over to the positive ion to make up its deficiency. This kind of union is of electrical nature and is readily broken by the action of water and other solvents. There is, however, another kind in which what may be regarded as more purely chemical forces are involved. This is especially to be seen in the compounds of carbon. More recently, Langmuir has put a more definite interpretation on the latter kind of combination. He gives the name "electro-valency" to the forces responsible for the first kind of reaction, where electrons pass from one component to the other; and "co-valency" to the second, where it is held that certain electrons belonging to one atom are taken over in common by the other, so that they form common property of both atoms. As mentioned, this is especially characteristic of organic compounds.

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A somewhat remarkable result follows from the work of the Braggs on the structure of crystals. They show that in a crystal of sodium chloride, for example, each

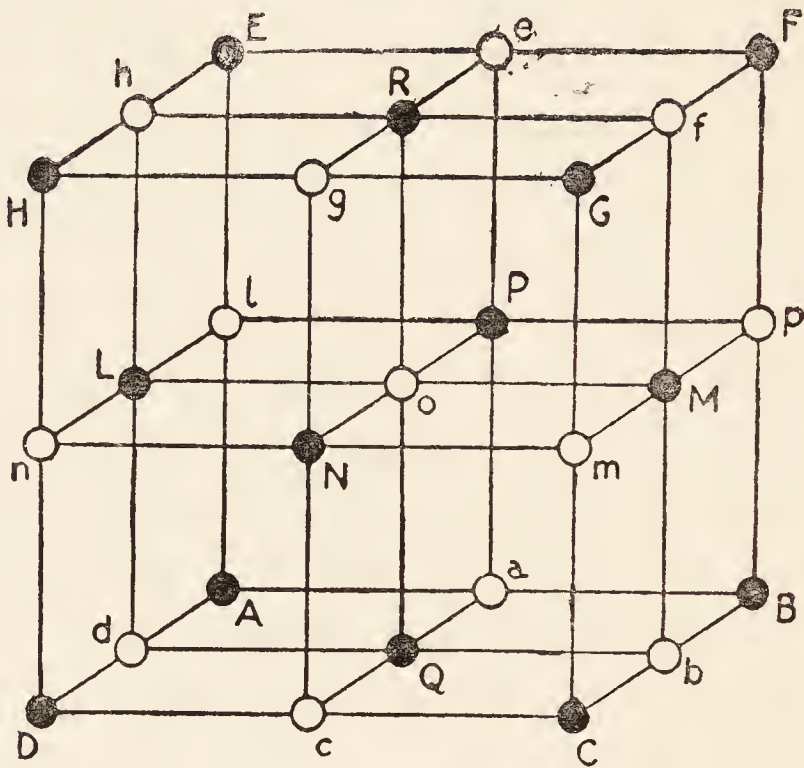


FIG. 1. Model of Crystal of Sodium Chloride.
Black—Na atoms. White—Cl atoms.
(Bragg's "X-rays and Crystal Structure.")

sodium atom is joined to six chlorine atoms, and each chlorine to six sodium atoms (Fig. 1). Hence there is no molecule of NaCl in the solid. Further, if it is completely dissociated in solution,

as is held by some, the only state in which it exists is in the vapour, a rare occurrence. We shall see later a somewhat similar problem is met with in colloids.

It is interesting to note that the electrical forces which hold together the atoms in a crystal are identical not only with the chemical forces responsible for the combination of sodium with chlorine, but also with those of molecular attraction which show themselves as cohesion and as internal pressure in liquids. In the case of liquids, the state of tension of a free surface is due to this attraction.

The importance of the considerations of the previous paragraph is that they show the futility of attempts to distinguish between chemical, electrical, cohesive and surface tension theories of such phenomena as those of adsorption.

The structure of the crystal has an aspect to which attention may here be called. The atoms at the surface are clearly combined differently from those in the interior. While each of the latter in the case of

sodium chloride is united symmetrically on all sides with six others, at the surface it can only be united with five. Thus, it has what may be called a potentially free "valency" and may take on another atom from a solution or gas in contact with it. This is the theory of adsorption put forward by Langmuir, to which we shall return later.

Phase

As a matter of convenience, it has been found useful to give a name to the separate non-miscible components of a heterogeneous system and that of "phase" suggested by Willard Gibbs has been generally accepted. We owe also to him the formulation of the well-known "phase-rule," which expresses the conditions of equilibrium in heterogeneous systems.

An adequate simple definition of phase is not easy, although as a rule there is no difficulty in recognizing it in practice. It

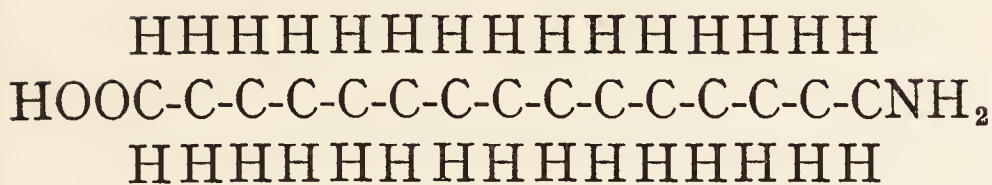
is not sufficient to say that it is a different physical state, as solid, liquid or gas. Water indeed may exist in a system of three phases, ice, water and vapour. But two liquids, as oil and water, may be distinct phases. Again, it has been suggested that the phases should be capable of separation by simple mechanical means. This would make colloids heterogeneous systems because they can be separated into their two component phases by filtration through a membrane whose pores are of such dimensions that the colloidal matter is unable to pass through. This would probably be a correct conclusion. But we are then met with the fact that if we make the pores still smaller, as in a so-called "semi-permeable" membrane, we can separate the components of an undoubted homogeneous system, as a solution of sugar or salt in water.

We seem to be reduced to the necessity to making the possession of surface properties a criterion of the right to be called a phase. We may say that the molecules

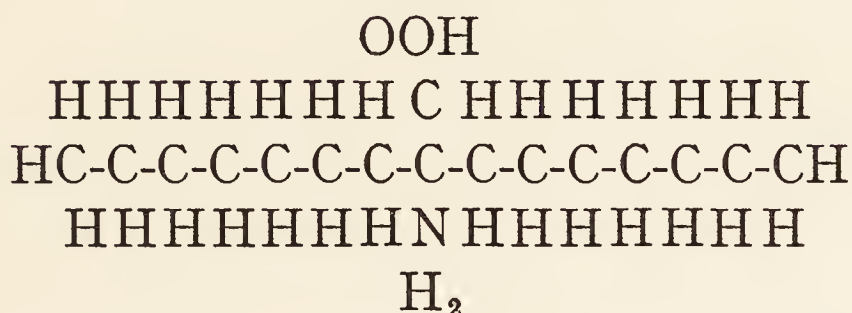
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in the interior of the phase are exposed to forces different from those on the surface, or, otherwise, that in homogeneous systems all the molecules are equally accessible to external influences, chemical or physical, whereas in heterogeneous systems only those on the surface of each phase are so accessible. But while this applies to the majority of forces, it may be said that heat affects the whole of a phase at the same time, or practically so. However this may be, the important point is that, as concerns chemical behaviour, only a part of the total mass present is capable of reaction at any particular moment. As was stated above, the active mass is a function of the total actual surface. This of course applies also to any other phenomena, such as surface tension, adsorption or electrical charge, which are dependent on the existence of surfaces.

The question naturally arises as to whether a single molecule may not be large enough to show surface properties. Such a molecule as



has not the same chemical properties as



where the basic and acidic groups are close together. Thus, space relations and shape have their part to play and, from what we have seen of the nature of the surface of a crystal, it does not seem impossible that the surface of such a large organic molecule might present opportunities for the adsorption of atoms. The possibility has its importance in relation to the properties of proteins, as will be seen in Chapter IV.

CHAPTER II

SURFACE TENSION

THE two most striking manifestations of the special properties of surface or interfaces between phases are those of surface tension and of electric charge. The latter is sometimes regarded merely as reducing surface tension, but there are properties, analogous to the charge on ions, which are more profitably discussed on their own account, as will be done later. In the present chapter, a brief consideration will be given to surface tension.

When molecules are made to approach each other, as in the compression or cooling of a gas, a force of attraction begins to manifest itself at a certain very minute distance apart (about 10^{-7} to 10^{-8} cm.).

Within this distance the force is very great. It appears to increase inversely as the fourth power of the distance and thus agrees with electrical attraction between charges concentrated in points. It may therefore ultimately be of electrical origin, and we have already seen that the Braggs' work shows it to be identical with the chemical forces uniting the atoms of a crystal. It is generally known as "cohesion" and is responsible for the great resistance opposed by a liquid or solid to mechanical attempts to increase its volume, and is also familiar in the a factor of van der Waals' "equation of state," and in the case of liquids, in the "internal pressure" of Young and Laplace. The importance of the above considerations in regard to the way in which we look upon adsorption will be clear at a later stage.

On the other hand, molecules occupy space, so that when pressed very closely together, they begin to resist further approximation. By great pressure, however, liquids and solids can be diminished some-

what in volume. If it be assumed that the molecules touch one another under ordinary conditions, such compressibility implies a deformation. The volume occupied by molecules is allowed for by the b factor of the van der Waals' equation.

We may now consider the state of affairs at the free surface of a liquid in contact with a gas in the light of the two facts above mentioned. In the depths of the liquid, the molecules are exposed on all sides to equal attractive forces, so that there is no effective resultant in any one direction. This is not so at the surface, where the molecules are not exposed to attractive forces on the outer side. The result is that they tend to be drawn inwards by the unbalanced attraction of the other molecules of the liquid phase. Owing to the practical incompressibility of the liquid, they cannot actually move inwards, since this would mean a decrease in the volume of the liquid. The pull inwards is thus converted into a pull or tension along the surface, as may be realized by the tension

produced in a horizontal stretched cord by a weight hung upon it.

This is the *surface tension* as shown by the surface of a liquid. It will be clear that a gas cannot exert a tension of this kind, except in a minute degree, because the molecules are sufficiently far away from one another to be almost entirely outside the sphere of mutual attraction.

It is not easy to picture the corresponding phenomenon at the free surface of a solid. But since the forces of cohesion are present here, and of a very high degree of magnitude, there must be at the surface an internally directed component, which gives a high surface tension, although it cannot be measured directly in the same way as in liquids. Indirect measurements confirm the theoretical deduction.

The tension is manifested by all the elements of the surface, so that if we take a finite area of this surface, we can obtain work from it. Thus it possesses *energy*. Perhaps the simplest way to convince oneself that this is so is to take an ordinary

glass funnel, and immerse the wide end in soap solution so as to make a film. Closing the end of the stem with the finger, raise the funnel from the solution. The film remains stationary. But when the finger is removed from the orifice of the stem, the surface tension in the liquid causes it to reduce its area. This can only be done by rising until it reaches the narrowest part of the funnel. Since this is done against gravity, work is done by raising the weight of the film.

Suppose that the surface of a liquid instead of being flat is curved as in that of a drop. A little consideration will make it clear that there is a component of pressure directed inwards towards the concave side of the surface film. If a stretched cord be deflected, it is felt to exercise a pressure on the deflecting finger and the more so the further it is deflected. There is, of course, a disturbing factor in such a simple case, due to the increase in tension as the cord is increased in length. But this could be eliminated by arranging the cord

to be stretched horizontally by a weight attached to one end after passing over a pulley. Or, more simply, by deflecting sideways a vertical cord stretched by a weight at the lower end. The pressure exerted in the interior of drops of the size of those in colloidal solutions by the tension of their surfaces is very great. If it be calculated for drops of water of the size of certain particles of colloidal gold, it is found to amount to some 150 atmospheres. Such pressures must have an influence on the properties of minute spheres. Very minute drops of liquid resist deformation as if solid. Thus they do not pass through pores smaller than their diameter. On the other hand, Sir Wm. Bragg has not found indications that the atoms of gold particles are pressed more closely together.

When a capillary tube is dipped at one end into a liquid which wets it, the level of the liquid is seen to be higher in the tube than outside. This is due to the formation of a meniscus which is concave on the air

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side, owing to the formation of a thin film over the inner surface of the tube above the meniscus itself. The surface tension of this film draws up the edges of the meniscus and thus brings into play a component force pulling up a column against gravity. This behaviour may be used as a method of measuring the surface tension of liquids, but needs precautions to get accurate results. More especially is it necessary before making a reading that the whole length of the tube be wetted by the liquid. This may be done by causing it to flow through for a moment.

Another method used to measure surface tension indirectly is that of the size of drops. The size to which a drop of a liquid issuing from a small orifice attains before falling off depends on the tension of its surface, which resists the action of gravity. Of course, in comparing different liquids, their specific gravities must be allowed for. Further, when a solution, and not a pure liquid, is being tested, the drop must be caused to form quite slowly. This is

because solutes tend to concentrate in the surface film by the process of adsorption to be discussed later, and time must be given for diffusion from the interior of sufficient amount to make the process complete. Otherwise, the readings will not be in agreement with one another.

In the determination of the surface tension of solutions, we see that the methods described above (and the same statement applies to the direct method in which a wire ring or a glass slide is pulled out of the surface by a measured force) determine what may be called the "static" surface tension, that is, the value when the surface condensation of the solute has become constant. If that of the solution itself apart from this factor is required, what are called the "dynamic" methods must be used. Such is the method of Rayleigh, in which the length of waves is measured. Hartridge and Peters have recently improved this method (Proc. Roy. Soc., A. 101, p. 348). The object aimed at in these methods is to measure the tension

of a surface which is continually being renewed, so that no time is given for the surface condensation to occur.

The direct method above referred to, in which the torsion of a wire required to balance the surface tension is determined, has been improved by Lecomte du Noüy of the Rockefeller Institute and by his apparatus very rapid measurements of a number of fluids can be made.

The effect of the majority of solutes is to lower the surface tension of a pure liquid. At an air-water interface, however, certain inorganic salts in somewhat high concentration are found to raise it. This is probably due in part to the fact that the solvent as well as the solute is condensed in an interface. If the solvent is more highly adsorbed than the solute, the surface film will be *less* concentrated than the main body of the solution. Further reference will be made to this so-called "negative adsorption" in a later page.

Since surface energy tends to a minimum, it is obvious that the drops of a liquid take

a spherical shape, so far as this is possible. The area of the surface of a sphere is less than that of any other figure of an equal volume. It follows from this that energy is required to change the shape of a spherical drop, because any other form has a greater surface area and therefore more surface energy. A point sometimes overlooked is that the surface tension per unit length in any given liquid is always the same whatever the area this liquid is made to cover. Thus the tension in the wall of a soap bubble does not change when the bubble is blown larger ; hence the pressure inside it decreases owing to the diminution of curvature, and that inside a small bubble is higher than that in a large one, so that, if joined together by a connecting tube, the smaller bubble empties itself into the larger one. In this way, a soap bubble differs from a blown-out india-rubber ball, in which the internal pressure increases as the distension becomes greater.

When two different liquids are in contact, the tension on the two sides of the inter-

face will very rarely be the same. Thus the surface will curve towards one side or the other. The fact has some important consequences. Clowes showed that sodium and calcium soaps had opposite effects in solution in oil and in water. A solution in water of a sodium soap has a lower surface tension than when dissolved in oil. Therefore the interface between such solutions becomes concave on the oil side and an emulsion of oil in water is formed. A calcium soap, on the other hand, has a greater tension in water than in oil. Therefore the interface is concave on the water side and an emulsion of water in oil is formed. This reversal of the internal and external phases has its bearing on the changes of permeability in the cell membrane, as will be seen.

The difference in surface tension on opposite sides of the interface is also the cause of the "*intertraction*" phenomena described by Almroth Wright. Serum passes into a layer of physiological saline on the top of it in opposition to gravity

and at a rate considerably greater than that of simple diffusion. An emulsion is not produced, of course, because the liquids are freely miscible. The possibility of the phenomenon appears to have been predicted by Clerk Maxwell as a result of relative surface tensions, and it has been shown by Schoneboom to be a general occurrence common to all solutes in any solvent. Taking the case of serum and saline, the surface tension of the former is lower than that of the latter, so that the interface is concave towards the saline, which would, if immiscible, tend to be emulsified in the serum. Being miscible, it is carried downwards into the serum and the serum rises upwards to take its place.

Other Forms of Surface Energy

The existence of electric charges at interfaces has already been alluded to and will receive further consideration in relation to adsorption. It is also to be remembered

that chemical reactions may also be taken part in by the molecules of the surface. It has been pointed out above that the whole mass is not concerned, so that the rate of reaction is not that of a simple mass action, unless the mass is of so porous a nature that all its molecules are equally accessible to the solution in which it lies. This is held by Loeb to be the case with proteins, but it is otherwise a doubtful hypothesis and requires proof. In the ordinary case, the course of the reaction will clearly be different according to whether the products of the reaction are soluble or not. In the former case, the whole of the solid phase will ultimately be dissolved, as zinc in sulphuric acid. In the latter case, an insoluble film will be formed, which hinders further access of the dissolving reagent, as calcium carbonate in oxalic acid.

In any case, it must not be supposed that all the peculiarities of interfacial phenomena are to be explained on the basis of a simple mechanical surface tension

of the usual kind. Hasty statements of this nature have led to some misunderstanding.

Before proceeding to discuss the physiological phenomena in which interfacial forces play a prominent part, attention may be called to the propensity in certain quarters of assuming the existence of special chemical compounds to account for every new activity observed. This should not be done until the compound has been shown to exist and its properties investigated. Names are given to these imaginary substances, although the names merely serve to state the fact. More knowledge of the possibilities of reactions in heterogeneous systems will make many of these names superfluous and obsolete. Ehrlich's "side-chain" theory, with its elaborate terminology, has largely disappeared, although it has served a good purpose in suggesting problems for investigation. Le Dantec has made the satirical suggestion of calling the various imaginary chemical compounds referred to "pheno-

menins.” Their assumption merely serves to hide ignorance and leads to no advance in knowledge. It may be admitted, however, that a name for a particular phenomenon sometimes has a good purpose in enabling fewer words to be used in description of it. But these names should never be given such a form as to suggest definite chemical individuals, until they are actually shown to exist.

It is proposed in the succeeding pages to consider first of all two general bodies of phenomena which are met with more or less universally—adsorption and the colloidal state. It will only be possible, however, to discuss certain aspects of these which have come into some prominence in recent times. Following this, some special physiological phenomena will be looked at from the point of view of the facts brought out in the more general discussion.

CHAPTER III

ADSORPTION

Theories and Explanations

THE question of adsorption is still a somewhat vexed and contentious one. There is no doubt that on the surface of a solid phase in contact with a liquid or gaseous one, or at the interfaces of liquids with each other or with gases, there is a condensation of the liquid or gas. If the liquid contains a solute, it usually happens that this solute attains a higher concentration in the interface than in the body of the solute. But the reverse may happen; the solvent may be adsorbed more than the solute, so that the concentration of the solute in the body of the solution may

rise. While there is no denial of the fact that charcoal takes up various gases, or coloured substances out of watery solution, if any suggestion is made that any forces other than those involved in combination in molecular proportions and dealt with in the theories of "classical" chemistry are concerned, a certain school of workers will have none of it. It is a curious fact that the subject seems for some reason to cause writers on it to make use of satirical and contemptuous language in referring to the views of their opponents. I fear that this charge has to be made chiefly against those who uphold purely "chemical" views. It will be well therefore to mention a few cases which seem to require the intervention of forces of a different nature, or acting in a different way, from those involved in ordinary combination in stoichiometrical ratio.

When colloidal silica is brought into equilibrium with water vapour at different tensions, it was shown by van Bemmelen that to every tension there corresponds a

different value of water content of the solid phase. This is in striking contrast to what happens with sodium sulphate. Figure 2, in a diagrammatic form, represents the

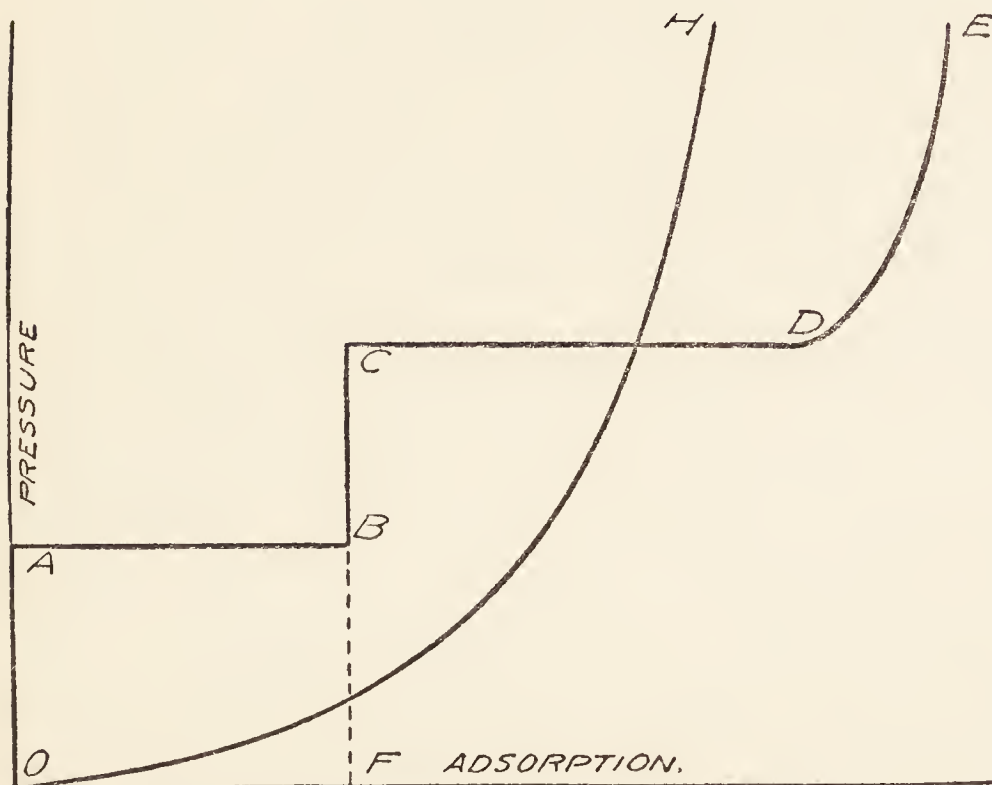


FIG. 2. Adsorption and Chemical Combination.
 (Bancroft: "Applied Colloid Chemistry."
 McGraw-Hill Co.)

two cases. The ordinates represent the pressure of water vapour and the abscissæ the amount of water taken up by unit mass of the solid. The curve OH represents

the case of silica, and is known as the typical "adsorption isotherm," since the temperature is supposed constant. The curve OABCDE is that of sodium sulphate. Along the line OA the solid phase is anhydrous; no water is taken up. Along the line AB the pressure remains constant until enough water has been taken up to convert the sodium sulphate entirely into $\text{Na}_2\text{SO}_4 \cdot 10 \text{H}_2\text{O}$. Along BC the pressure continues to rise, but nothing happens until C, at which point more water begins to be taken up until a saturated solution of sodium sulphate is formed. Along DE the saturated solution takes up water continuously. The characteristic of the adsorption curve is the steady continuous change with rise of concentration; the steps due to definite chemical compounds are absent.

Charcoal takes up various gases in different amounts, at low temperatures even the inert gases, argon, neon, etc. If chemical combination in the usual sense were the explanation, it is difficult to see

why argon at low temperatures should be "adsorbed" to practically the same extent as nitrogen or hydrogen. Further, as Bancroft points out, if the adsorption of chlorine by charcoal is a chemical combination, why is it so difficult to obtain compounds of carbon and chlorine by direct action?

Another curious case is one that I met with in some work on the properties of Congo-red. It is easy by dialysis to obtain a colloidal solution of the free acid of this dye, which is itself a sodium salt. This acid is of a blue colour, whereas its salts are red. If a colloidal solution of the hydroxide of aluminium, thorium or zirconium, free from acid or alkali, is added to such a blue solution, an insoluble blue complex of the two colloids is formed. If this blue "compound" is warmed, true combination occurs with production of the red salt. The change also occurs slowly at room temperature. The precise explanation of the facts is difficult. It may be that the insoluble blue acid is a

“pseudo-acid” and is converted into the true acid, which is stronger, by heat. But this does not affect the circumstance that some sort of association by contact takes place, without chemical combination. Similar phenomena may be seen with porous earthenware and with silk, in the presence of calcium sulphate, when these solids are taken in place of the hydroxides. It will be seen presently that the necessity for the presence of the electrolyte in the case of silk is because the blue acid has a negative electrical charge. The hydroxides and the porous clay are positive, so that their electric charges do not repel. Silk has a negative charge and repels the acid, unless its charge is neutralized by the positive bivalent calcium ions.

Admitting the existence of adsorption, as I think we must, what is the explanation of the way in which it is brought about? What are the forces which come into play? It must be admitted that up to the present no simple satisfactory or general theory has been put forward. It is probable that

various factors are concerned, both chemical and physical, one or the other being predominant according to circumstances. An exclusive adherence to either of these alone presents difficulties.

The thermodynamical treatment of adsorption adopted by Willard Gibbs is familiar to all. We have seen that most solutes depress the surface tension of the liquid in which they are dissolved. If they become concentrated in the surface they will have more effect in this way. Now the surface film possesses free energy and the second law of energetics tells us that free energy always decreases, if it can. Thus we expect to find concentration of the solute in the interface. Gibbs has further proposed a mathematical expression on the basis of surface tension of the mechanical kind. Experimental work has shown that there are some cases which follow the Gibbs formula, while in other cases more solute is adsorbed than corresponds to the amount expected. It appears that these cases which do not follow

the formula are such that other factors than mechanical surface tension may be supposed to be present. They are electrically charged colloids or electrolytically dissociated substances. Perhaps aggregation of colloids may also play a part. It has been pointed out by Wolfgang Ostwald that Gibbs' application of the second law of energetics to adsorption is to be extended to surface energy of any kind, whether mechanical, electrical or even chemical. But, like all thermodynamic reasoning, although the conclusions as to what must occur are always cogent, we are not given any information as to the mechanism of the process. We have therefore to inquire what hypotheses have been suggested as to this mechanism.

Prof. Matthews in an interesting article in "Physiological Reviews" advocates forces of "cohesion" or attraction between molecules as the cause of adsorption. We have seen that in the case of liquids cohesion is identical with the classical Young-Laplace "internal pressure," which is the

cause of surface tension. When dealing with solid surfaces, it is doubtless preferable to speak of "cohesion," rather than surface tension. But it is difficult to understand why a distinction is made between surface tension and cohesive forces.

A consideration of certain statements made by Matthews will give us opportunity for the discussion of other views on adsorption. As we have seen, there are certain cases which require some mental gymnastics to account for on a purely chemical basis. Whatever happens after adsorption has taken place, no sufficient proof has yet been given of chemical forces capable of *bringing about* a greater concentration on an inert surface than in the body of the solution. This will be more evident after we have examined the views of Langmuir.

Matthews says that "cohesion and chemical affinity are not the same." It has been already pointed out above that the Braggs' work on crystal structure has made it pretty clear that they are identical,

at all events in crystals. The question may well be asked—Are we justified in attempting to distinguish between chemical and physical forces in the atomic and molecular region? There is no real opposition between the two points of view. Why should there be such violent attempts to prove adsorption to be exclusively one or the other? The important thing is—What is it? An explanation which serves for a few facts only is an incomplete one. Some phenomena may be easily explained on a purely chemical basis of the ordinary kind, others not at all or only by giving an unusual meaning to the name “chemical.” There is no doubt that there are cases, such as that of water of crystallization, which serve as transitions between what are chemical phenomena in the strict sense and physical attachment. And it seems to the writer that to insist too strongly on the chemical nature even of water of crystallization tends to obscure and minimize the essential characters of chemical combination, where the chemical properties

of the compound are greatly changed. Water behaves as a chemical reagent very differently from hydrogen and oxygen. For example, it combines with P_2O_5 to make H_3PO_4 . Hydrogen and oxygen will not do so. There can be no doubt that true chemical combination is associated with changes in the internal mechanism and structure of the atoms concerned. In this process, energy is given off or taken in. Although heat is given off in the process of adsorption—a fact not to be overlooked—the source of this energy is the compression involved in the condensation, the molecules being pressed closer together. It is similar to the heat evolved in the compression of a gas by a pump. Of course, we expect ultimately to express chemical facts in terms of atomic structure, but for the present there is a place for a science of chemistry, with its own laws. The position is similar to that of biology. Although we may believe that its data will sometime be expressed in physico-chemical language, for the present we must be con-

tent with a body of doctrine which consists for the most part of laws only capable of expression in a terminology peculiar to vital phenomena.

To return to the problem of adsorption. When Matthews states that it is not certain that a capillary-active substance will concentrate at a solid-liquid or liquid-liquid ("dimeric") interface, the reply may be made that if "capillary-active" means "depressing surface energy," a failure to concentrate would contravene the second law of energetics, a thing which does not happen in the present world.

It is difficult to understand in what sense we are to interpret the statement that "chemical and physical dissociations follow the same isotherm." No cases are given, but we have seen that this is not always so. We must remember that we are dealing with heterogeneous systems where the phase rule applies. A typical case of chemical dissociation is that of calcium carbonate, worked out by Le Chatelier. Expose this solid at a temperature of 745° to

a current of carbon dioxide at atmospheric pressure. No change occurs. Gradually reduce the pressure. Still there is no change until the pressure falls to 29 mm., when the *whole* of the carbon dioxide is given off from the carbonate and the oxide remains. There is no gradual process. We shall see later the application of this behaviour to the dissociation of oxyhæmoglobin.

A characteristic of adsorption is that its degree is proportional to each definite concentration in the liquid phase. If this concentration is lowered, some of the adsorbed solute comes away. This is regarded by Matthews as also a property of chemical compounds. It is however only true when a compound is already dissociated by hydrolysis. In this case, if the acid or base is more soluble in some other solvent, shaking with this solvent will separate the two constituents. But it is not the presence of the new solvent that causes the dissociation. The phenomenon only occurs in hydrolytic dissociation.

Although hydrochloric acid is soluble in benzene, sodium chloride is not decomposed by shaking a watery solution with benzene.

We may now turn to the views of Langmuir, who certainly gives an explanation of some cases of adsorption. Those to whom it gives comfort may call his theory a chemical one, but it is so in the same sense as cohesion is. As Sam Weller said, "it depends on the taste and fancy of the speller."

The cases in question are those in which ions are adsorbed by crystal surfaces. We have seen that the surface atoms have what might be called "potentially free valencies." They occupy definite points of the lattice and may combine chemically with ions of opposite sign. Thus the sodium atoms in a crystal of sodium chloride may combine with anions present in a solution in contact with the crystal. The chlorine atoms may combine with cations. It will be seen that the adsorbed layer cannot be more than one molecule thick, and this is often the case.

On the other hand, it appears that the layer on non-crystalline, amorphous, surfaces may be thicker. It has been suggested that solid solution in the mass of the adsorbent may take place, but this is merely hypothetical. The allied question of imbibition will be discussed later.

With regard to these inert amorphous bodies, we may justifiably ask for evidence of chemical combination between charcoal or carborundum and saponin or amyl alcohol.

Orientation

The views of Langmuir in this connexion are of much interest and are more strictly chemical. Suppose we take the case of adsorption of a fatty acid, such as butyric acid on the surface of carborundum or kaolin. If we hold that the adsorbed layer cannot be more than one molecule thick, it is clear that the number of molecules that can be packed on will depend on their position, if they are thicker in one

direction than in another. In the case of a long chain acid, the amount adsorbed is greater than could be accommodated if the molecules were lying flat on the surface. It must therefore be supposed that they stand upright. Since the carboxyl group has a greater affinity for water than the hydrocarbon group has, it is reasonable to expect these ends of the acid to be directed outwards into the water phase. Other cases might be given, such as that of benzene, where the rings appear to lie flat until combined with some group having an affinity for water. In this case, the rings are turned edgewise in order to direct this group towards the water. When we come to speak of enzymes, it will be seen that these views have important implications. But while the affinity of carboxyl for water may be accepted, it is not so easy to suppose that the hydrocarbon chain has affinity for kaolin or charcoal. Thus the arrangement described above is in all probability present *after* the molecules have condensed on the surface. But it is not so clear what forces

cause the concentration on the surface to be greater than in the main body of the solution. It remains to be shown what it is that holds a molecule on the surface when it has no chemical affinity for the material of this surface. The movements of the molecules in the liquid phase bring them into contact with the surface, but if they are to remain there and so become concentrated, it seems that some kind of force must hold them.

On the whole, while Langmuir's theories account satisfactorily for some particular cases, further development is necessary in order to form a general theory of adsorption.

It seems possible that some evidence might be obtained of the formation of compounds with naphthalene or other substance, which though insoluble in water is soluble in some other liquid. In true solution, it should form more of the compound than when in suspension in water.

Electrical Adsorption

In the discussion of this aspect of the problem of adsorption, it will probably be best to describe first certain facts in connexion with dyeing and staining and then to consider the explanation of these facts. I believe that I was the first to call attention to the relation of this process to electrical charges, although the basal phenomenon as regards colloids had been pointed out by Victor Henri and Languier des Bancels at an earlier date than my paper, which was unfortunately published in the "Biochemical Journal" in 1906. This journal was not widely known at that time and my work has been lost sight of. Being engaged on some problems in connexion with the intervention of adsorption in the action of enzymes and wishing to investigate a simple case in which the chemical nature of the reacting substances was known, I took Congo-red as taken up by filter-paper.

To my surprise I found that the purer the

paper, that is, the less ash it contained, the *less* dye was adsorbed. The addition of a neutral salt, even sodium chloride in small amount, caused an increase. Further experiments showed that it was the cation of the salt that was responsible and that bivalent ions were very much more effective than univalent ions. The anion was almost without effect; what there was appeared to be opposite in direction from that of the cation. Other so-called "acidic" dyes, that is, neutral salts in which the coloured component is the acid or anion, gave similar results.

The case was different with "basic" dyes, in which the coloured constituent of the salt is the base or cation. Here, the purer the paper, the *more* dye was adsorbed, while electrolytes depressed the effect. Again it was the cation of the salt that was concerned.

At this point, it is necessary to refer to a recent paper by Michaelis and Rona (1920), which is quoted by Matthews in support of his contention that "the adsorption of

electrolytes is due to chemical forces." These authors state that the adsorption of basic dyes is due to their chemical combination with the ash of the paper. My contrary result, that the less salt the greater the adsorption, is so easy to obtain that I use it as a regular class exercise, and it is quite beyond my comprehension how careful observers could have obtained any other result. In any case, Matthews' argument falls to the ground.

Michaelis and Rona state further that it is because the calcium compounds of the dyes in general are insoluble that they are fixed in the paper. But sodium-ions are very effective with Congo-red and the sodium salt in this case is soluble.

It is interesting to note, however, that these observers find that there is true adsorption, apart from chemical combination, in the case of charcoal.

I pass on to the explanation of the facts concerning the dyeing of paper. What V. Henri and L. des Bancels found was that Congo-red or aniline-blue did not readily

stain gelatin. It was also found that both gelatin and these dyes possess negative electrical charges and therefore repel one another. If they added barium nitrate, the gelatin became deeply stained. The explanation given was that the barium ions neutralized or reversed the charge on the dye, so that the obstacle to the attachment to the gelatin was removed. Let us see how this applies to our case. I may note, to begin with, that in my original paper I adopted the hypothesis that the electrolyte reverses the charge on the *dye*, but the form of the explanation given in the following lines is more in accordance with other facts, as will be seen.

Origin of Electric Charge on Surfaces

Quincke found that most chemically inert and insoluble bodies possess a negative charge in water. This was confirmed and investigated further by Perrin. It appears that this charge may have a different

origin in various cases, and it is to be remembered that the considerations brought forward apply not only to surfaces of visible dimensions, but also to those of colloidal particles.

It was pointed out by Coehn that when two substances of different dielectric constant are brought into contact, that with the lower constant becomes negative to the other. Water has a higher dielectric constant than paper, so that we should expect the latter to be charged negatively. This fact is connected with the phenomena of frictional electricity, and it is possible that the negative charge on drops of paraffin in water, as shown by Lewis, may be due to friction as they move through the water.

Another cause of electric charge is the adsorption of ions, owing to their reduction of surface energy of some kind. It will be clear that a layer of cations on a surface will give it a charge of positive sign ; of anions a negative charge. Perrin showed that the sign of the charge on many surfaces can be

reversed by the action of acids or alkalies, as the case may be. Paper, however, is refractory to acids, the negative charge being diminished, but not reversed, by $m/30$ hydrochloric acid.

A third means by which a charge may be given to a surface is electrolytic dissociation of the material of the surface itself. The molecules of the surface of a particle of silicic acid dissociate into hydrogen-ions and silicic anions. The former being soluble tend to diffuse away into the surrounded water, while the latter being insoluble remain on the surface, giving it a negative charge. But owing to electrostatic attraction, the former cannot get further than a very short distance away from the opposite ions. Thus there is formed a Helmholtz double-layer. Aluminium hydroxide gives off OH-ions and is left with a positive charge. The colloidal particles coated with ions in this way are called by Hardy "colloidal ions."

Ferric hydroxide is interesting. As prepared in the usual way in presence of

excess of ferric chloride, the particles have a positive charge, possibly owing to electrolytic dissociation of the surface or to adsorption of hydrogen-ions. But, as Powis showed, if precipitated by excess of sodium hydroxide, it has a negative charge. It is difficult to explain the facts otherwise than as due to adsorption of OH-ions. If the negative ferric hydroxide were a sodium ferrate or ferrite, it would possess an osmotic pressure, which I find to be absent.

Turning now to the nature of the dyes used, we note that they are mostly electrolytically dissociated salts. In the "acidic" dyes, the anion is a complex organic coloured compound, the cation is usually sodium. In the "basic" dyes, the cation is the complex coloured compound; the anion, usually chlorine.

Imagine these in the presence of negatively charged paper. The colloidal negative ion of the acid dye is repelled by the similar charge on the paper. If deposited, it would raise the free energy by

increasing the charge, and this is contrary to the second law of energetics. When the charge on a surface is actually increased by adsorption of ions, the energy required is afforded by some other source, such a surface energy of the mechanical kind. On the other hand, the positively charged coloured cation of the basic dyes is powerfully attracted by the negative paper and reduces the charge.

Harrison has shown that there is an optimum temperature for the adsorption of dyes, at which the amount taken up is the maximum. It was found that at this temperature the electric charge was also at its maximum. The reason for this effect of temperature on the charge is not clear.

Now Perrin showed that the charge on surfaces can be reduced or reversed by electrolytes. Since our paper surface is negative, it is the cation of the electrolytes that is active—the anion would raise the free energy. When the charge on paper is decreased or reversed, an acidic dye can be adsorbed, but that of basic dyes is

retarded, as we found. The electrolytes in question may be added, or they may already be present in the paper if ordinary filter paper be used.

We may note that the process is not chemical in the proper sense. Heating the system, however, causes combination, which takes place slowly even at room temperature. When this has taken place, the dye cannot be removed by soaking in water, whereas this can be done when the stain is fresh and not heated. The simplest way to show this is to place a dyed piece of paper in water along with an unstained piece. The latter gradually becomes stained.

An interesting point is that the addition of alcohol increases the adsorption of an acid dye, decreases that of a basic dye. The explanation of this behaviour is obvious. The negative charge on the paper is diminished by alcohol because of its lower dielectric constant than that of water. The decrease of electrolytic dissociation may also play a part. The use of alcohol for

removal of dye in histological differentiation is a common practice.

When an acidic dye has been adsorbed by paper under the influence of salts, it is very difficult to remove it by washing with water. The charge having been neutralized by the adsorption of ions, these ions are held by electrical forces. To enable them to be removed, something must be done to restore the original negative charge of the paper. It is sometimes possible to do this with alkalis, but the difficulty is that there is always the cation of the alkali present to oppose the effect.

It is a curious fact that the phenomena described seem to happen only with colloidal dyes (or large molecules). Staining with picric acid is unaffected by electrolytes. Perhaps a true chemical compound is formed by this somewhat powerful reagent. If the dye is an electrically charged colloid but not a salt, such as "Scharlach R." a so-called "indifferent" dye, which does not form salts with either acids or bases according to Michaelis, it is affected by salts in the

same way as congo-red is. The fact that this dye stains paper according to rule, although it forms no salts, places a difficulty in the way of interpretation of dyeing as a form of chemical combination. In the presence of gelatin, the effect of electrolytes is practically absent, just as in the case of colloids. Presumably the paper is coated with a layer of emulsoid insensitive to electrolytes. Complex conditions are present when the protective colloid is itself a salt with an acid or base. In the former case, the effect of the electrolytes is increased; in the latter, decreased. No simple explanation is forthcoming.

It is rather remarkable that hydrogen and hydroxyl-ions have comparatively little effect on the adsorption of dyes by paper. This also needs explanation.

Matthews, in the article referred to above, objects to the introduction of electrical charge into the theory of adsorption on the ground that the union of sodium- and chlorine-ions should then be called adsorption. On this point, we may remember

that modern theory, advocated chiefly by G. N. Lewis, makes it most acceptable to adopt the view that strong electrolytes in solution in water are completely dissociated. Thus, sodium chloride is not present as such in solution, only its ions not in combination exist there. Further, sodium chloride has totally different chemical properties from those of its constituents, whereas dyed paper has not. The fact of heterogeneity is also overlooked. The electric charge on a surface may be other than ionic in origin. The fact of fixation in some way is the important point.

Dyeing and Staining in Histology

The facts referred to above have an obvious bearing on the theory of dyeing and staining. There are many factors entering into the varied and elaborate technique of the histologist, but the meaning of these is not yet clear. We may call attention to some points which arise from what has been

said above and to others which indicate the inadequacy of some current views.

We have seen that the work of Perrin shows that in alkaline media inert solid surfaces take on a negative charge. This seems to be due to adsorption of hydroxyl-ions. When these structures are of protein nature, it is likely that the electrolytic dissociation of metallic salts of protein (in the cell, usually of sodium, potassium or calcium) by which an insoluble protein anion remains on the surface, plays an important part. The reaction of the cell contents is normally on the alkaline side of that of water and certainly of the isoelectric points of these proteins. Other inert structures, such as those of carbohydrate or lipid nature, will also have a negative charge by adsorption of hydroxyl-ions, even if electrolytic dissociation is absent. We may reasonably expect that the behaviour of such constituents to dyes and the effect of electrolytes upon them will be in accordance with the phenomena with filter paper. This appears to be the case in the main.

The fixing action of heat and the "differentiating" action of alcohol are also similar. The hypothesis has been put forward that dyes form definite salts by double decomposition when they stain protein structures. Martin Heidenhain finds that a protein stains red with the blue acid of Congo-red, which certainly looks like formation of a salt. The puzzling thing about it is that unlike the known salts of the acid, the "compound" with protein is not affected by 5 per cent. sulphuric acid. There is involved some factor as yet obscure. Again it is stated that the object of using mordants is to produce an insoluble double compound of the dye, the mordant and the tissue. These supposed compounds are called "lakes" and, like the Congo-red compound, are not decomposed by acids and alkalis when they constitute a part of a stained cell. Although barium forms insoluble salts with most dyes, it is stated to be ineffective as a mordant, whereas the trivalent aluminium is in common use.

A complete theory must of course take into account “*specific*” staining or adsorption of dyes. It is to be expected that the electro-negative components of tissues would be deeply stained with “basic” dyes in general, and this is the case. But, as the common practice of washing with solvents shows, some of these structures hold certain dyes more firmly than they do others or than other structures hold the same dye. There are difficulties in accepting a purely chemical theory of special relationship to particular groups. Although the special affinity of methylene blue for nervous tissue is marked, other tissues are not devoid of it, while some dyes which are of quite a different chemical nature are also more or less specific nerve stains. Other factors must clearly be taken account of, such as density of electric charge and so on, but theory is as yet defective in this matter.

Concluding Remarks on Adsorption

On the whole there seems to be sufficient evidence to warrant the statement that substances can be concentrated at an interface by the action of forces other than those usually called chemical. Michaelis and Rona themselves find evidence of this in the case of charcoal. What the forces responsible for the preliminary fixation on the surface are is not clear. There appears to be a variety of them, although they may ultimately turn out to be essentially one. In some cases, chemical forces are responsible even for the preliminary part of the process ; in others, they may serve for orientation of the molecules in a particular way *after* adsorption. Chemical reaction with the material of the surface may also occur after adsorption, and, when more than one kind of molecule is adsorbed at the same time, these may interact with one another under the influence of the powerful compressive forces at the surface (heterogeneous

catalysis). But these are secondary phenomena as regards time.

The justification for speaking of a process as "adsorption" in the present state of knowledge is that the name does not, or should not, pretend to explain the process, but to call attention to the fact that there are factors in it which are not completely explained by the simple hypothesis of chemical combination in its ordinary meaning. In future discussion, however, we must keep in mind the identity of cohesion, chemical affinity and electrical forces in the crystal, as comes out in the work of the Braggs.

There is one point which should not remain unmentioned. No mathematical expression for the process has been given in the preceding pages. It appears that there is no satisfactory general formula for the whole course. Adsorption from comparatively dilute solutions follows the parabolic law of Freundlich, which may indeed serve so far as a criterion. But this formula takes no account of the fact that

above a certain concentration no more is adsorbed, the surface is *saturated*. This follows as a natural consequence of Langmuir's theory, and in any case the influence of the molecules of the surface cannot be expected to extend farther than a very limited distance. Wolfgang Ostwald suggests that some of these apparent cases of saturation may be due to simultaneous adsorption of solvent and solute in equal proportions, so that if the process be followed in the usual way by ascertaining changes in the concentration of the solution, there would appear to be no further change. There is no doubt that the adsorption of solvent is a matter of much importance and had been somewhat overlooked in early work.

CHAPTER IV

COLLOIDS

SO many problems are involved in the properties of the colloidal state that our attention must be confined to some few that require special mention at the present time or where a word of warning may not be out of place. There is a tendency to exaggerate the general importance of certain facts which are explicable best on a purely chemical basis, to the neglect of others which require in addition the introduction of physical factors, if they are to be interpreted without undue looseness of definition. Some writers appear to hold that there are no conditions peculiar to the colloidal state—that the properties of a particular compound are always the same, whatever its physical condition.

Thus there is some risk that a problem may be considered to be solved, when there yet remains a great deal to be found out.

“Classical” and “Colloidal” Chemistry

The opposition made by Prof. Jacques Loeb between what he calls “colloidal” chemistry and “classical” chemistry is a puzzle to me. At first, it seemed that theories relating to heterogeneous systems were to be excluded from application to colloids. But we find osmotic pressure and the Donnan membrane equilibrium freely made use of in Prof. Loeb’s work. It might indeed seem to some that the latter is strained to breaking point. Also, phenomena due to aggregation, while not looked upon with favour, are recognized. Finally, the only difference turned out to be the existence of adsorption, which is not to be mentioned in the discussion of colloids. We have seen reason to believe in its genuine occurrence where surfaces

are present, and these must be present if there is such a thing as aggregation of molecules to form particles. We shall see reason to accept this even in the case of proteins. The purely chemical properties can scarcely differ much, if at all, when molecules or ions are joined to form masses; but the physical properties do, and these are of much physiological importance. It is only necessary to mention viscosity, surface tension, osmotic pressure and so on, even if we omit adsorption.

These preliminary remarks suggest that we take proteins as the text of our discourse. They are of especial interest to the student of vital processes, although it seems to the writer that their importance as chemical agents is apt to be over-estimated. In order to make certain conclusions clear, it will be necessary to consider some other systems in relation to them.

Prof. Loeb seems to be under the impression that "colloidal" chemists in general deny that proteins combine with strong acids and bases and in apparently mole-

cular proportions. He would, however, find it a matter of difficulty to indicate more than one or two who take this view. At the same time, it is by no means easy to fix upon a definite molecular or combining weight for these complex compounds. They do not behave in the same way under all circumstances. Moreover, even admitting this view, we cannot explain

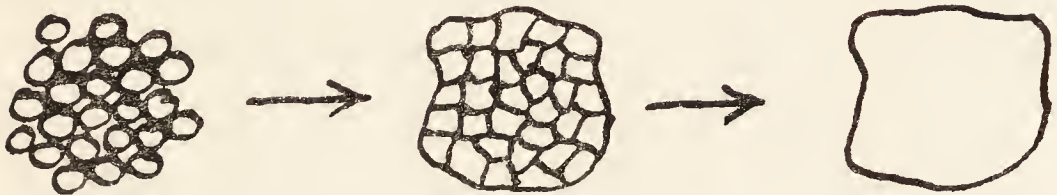


FIG. 3. Coagulated egg-white in three forms of aggregation.
(Chick and Martin.)

everything by it. For example, egg-white, as coagulated by heat in the experiments of Chick and Martin, is sometimes readily dispersed by electrolytes, sometimes not. The only explanation suggested is that of Figure 3, where the first figure represents the readily dispersed state, while the second and third, where the small particles have coalesced to form one large one, are only accessible to ions on the outside.

Aggregation and Osmotic Pressure

We may agree that in all probability there are molecular chemical relationships between proteins and acids and bases, when equilibrium has been reached. This, of course, presupposes that the original material is accessible throughout, if aggregated, to the action of the acid or base. But it is scarcely conceivable that the rate of action could follow the simple laws of mass action, because of the uncertainty of the concentration of active mass at any given moment. Even if ultimately brought into contact with the reagent, the internal molecules cannot be accessible as rapidly as those on the outside.

It is not intended hereby to cast doubts on the truth of mass action. But, when expressed in numerical laws of velocity of reaction, it is assumed that all the reacting molecules are free for kinetic migration and prediction of collisions by the theory of probability.

Let us consider, to begin with, the state of affairs in a case which is somewhat simpler than that of the proteins, namely, Congo-red. Since we know the constitution and molecular weight of this dye, which behaves as a colloid, it occurred to me that an investigation of its osmotic pressure would throw light on that manifested by more complex colloidal systems. These measurements showed a rather remarkable phenomenon. Although the electrical conductivity of solutions of various concentration showed the dye to be nearly as highly dissociated as sodium sulphate, the osmotic pressure was only that which would have been given if the molecules had been those of a non-electrolyte, such as sugar. Since the membrane used was of parchment-paper, which is permeable to the sodium-ions, an obvious explanation suggests itself at once, namely, that it is only the non-diffusible anion that was giving an osmotic pressure. This would have the same concentration as the molecules if these were undissociated. That this is not correct was shown in two ways.

The ordinary Congo-red is the di-sodium salt of a dibasic acid. But there is another dye, "Chicago-blue," of similar chemical constitution, being a quadri-sodium salt of a quadri-basic acid. Now if it were only the anion that is osmotically active, the osmotic pressure of equimolecular solutions of these two dyes should be the same. In point of fact, that of Chicago-blue is just about double that of Congo-red. Again, if the sodium-ions are inactive because the membrane is permeable to them, the osmotic pressure measured by a vapour-pressure method or by a membrane impermeable to sodium-ions, should be much higher than when parchment-paper is used. They were found to be the same.

A mathematical investigation of the problem also shows that the sodium-ions must be active. The reason why these ions do not escape is clearly because they are kept back by the electrostatic attraction of the opposite ions. But it is not so easy to see why they exert an osmotic pressure. The only way to arrive at an understanding of

the matter seems to be to think of the anions as being able to pull back the diffusible cations merely in virtue of having the membrane to put their feet against, as it were, to enable them to pull effectively. Thus this pressure, which is equal to the osmotic pressure of the sodium-ions, is borne by the membrane and indicated by a manometer connected with the interior.

The diffusion of sodium-ions to the outside of the membrane confers a positive charge there. The magnitude of this can be calculated from the Nernst formula for concentration cells. A similar process accounts for the electromotive force of certain living tissues and will be discussed later.

But there still remains the discrepancy between the electrical conductivity and the osmotic pressure of the dye solutions. If there were not something peculiar in these colloidal solutions, and absent from ordinary electrolytes in true solution, the osmotic pressure would have to be very much higher to correspond with the total ionic

concentration. The only way to account for this satisfactorily is by the suggestion that I made in 1911, namely, that the anions arising from electrolytic dissociation are attached together in large aggregates, probably owing to their large size and relative insolubility. The dimensions of these aggregates is supposed to be so great that their osmotic concentration counts for only a negligible fraction of the whole. But in order to satisfy the demands of the electrical conductivity, it must be assumed that these aggregates of anions possess the combined charges of their constituents and have a rate of migration at least equal to that of simple ions.

This conception has been found necessary in the case of the salts of proteins also; the protein ions like other large organic ions must be always aggregated. Thus we are led to the conclusion that proteins exist as aggregates not only at the isoelectric point, but even when in the form of salts. This being so, they always have surface properties.

The hypothesis put forward by myself in relation to Congo-red has recently been applied, with additions, by McBain to the case of soaps. After finding that molecular theories were untenable, he introduces the idea of the "ionic micelle." This is supposed to contain, in addition to ions, a certain proportion of undissociated molecules together with water. It may well be that the aggregates of anions of my original theory contain undissociated salt and water.

Wo. Ostwald refers to a fact which shows that certain dyes exist in large aggregates. Night blue in watery solution does not pass through parchment-paper, whereas in alcoholic solution it does. The particles cannot be smaller than molecules in the latter case, therefore they must be larger, that is, in the form of aggregates of several molecules, in the watery solution.

It will be remembered that Loeb makes great use of the Donnan membrane equilibrium as accounting for nearly all the properties of protein solutions. But it may be

pointed out that a somewhat arbitrary definition of colloidal behaviour is given. Moreover, the Donnan theory is not concerned with pure colloidal salts in equilibrium with water. Loeb, however, has not given much attention to such cases. Donnan and myself, independently, found that when there is an indiffusible salt inside a membrane, a neutral diffusible electrolyte also present is not distributed equally between the two sides. Donnan calculated thermodynamically the proportion of this salt on the two sides, finding that the inequality is the greater the higher the concentration of the indiffusible salt in relation to that of the diffusible electrolyte, but always such that there is more of it outside than inside. The result of this is that the apparent osmotic pressure of the indiffusible substance is lowered by the difference between those of the diffusible salt within and without the membrane. According to Sørensen, however, the fall in osmotic pressure of egg-white produced by the addition of neutral salts is not com-

pletely explained by this effect ; it is necessary to suppose that there is aggregation also. Thus, the effect of salts on proteins is similar to that which they have on inorganic, suspensoid, colloids.

Since there is still some misconception as to the part played by foreign electrolytes in the properties of proteins, it is well to point out that their osmotic pressure cannot be explained by the assumption that the effect of acids and alkalies is merely due to adsorption of ions and not to the production of dissociable salts. Such osmotic pressure may rise to quite a notable height, whereas a little consideration will make it clear that the attachment of an ion to a particle can only increase the mass of the particle, without raising the effective concentration. Thus, the osmotic pressure shown by certain colloids cannot be explained as being due to insufficient removal of impurities. Indeed, it is easy to show with Congo-red that the more effectively these impurities are dialyzed away the higher is the osmotic pressure.

The Isoelectric Point

The reference in the previous section to the formation of salts of proteins leads to some remarks about the conception of the "isoelectric point." The term is used very frequently at the present time and sometimes in a rather vague manner, without clear definition. Strictly speaking, it means an absence of electric charge, which absence might be brought about by neutralization of a charge of particular sign by the addition of any ions of opposite sign. It was originally used by Hardy in reference to the abolition of the charge on arsenious sulphide by a certain concentration of barium-ions. We have seen that the sign of the charge on an inert surface, as shown by Perrin, can be altered by hydrogen- or hydroxyl-ions. The matter has been thoroughly investigated by Loeb in the case of gelatin and other proteins. It will be realized that if gelatin combines with acids and with bases, there must be

some point between the two where it is not combined with either. Since the charged ions in this case result from the electrolytic dissociation of salts of the proteins, if such salts are not present, the protein will be devoid of charge. This particular concentration of hydrogen-ion, which is usually somewhat on the acid side of the neutrality of water, is called the "isoelectric point" of each protein. But it will be admitted that when the name is given a general application the ion referred to should be specified. We find that in the case of proteins at their isoelectric points, various properties such as viscosity, osmotic pressure, electrical conductivity and chemical activity are at their minimum values.

The actual position of the hydrogen-ion isoelectric point in the case of amphoteric electrolytes, such as proteins, depends on the ratio of their strength as acids to that of their strength as bases. These dissociation constants can be measured in various ways when we have no conflicting factors

to deal with. Thus they have been determined for a number of amino-acids, and the same methods should be applied to proteins. In the simple case the following formula applies :—

$$\text{Isoelectric point} = \sqrt{\left(\frac{K_a}{K_b} \cdot K_w\right)}$$

where K_a is the acidic dissociation constant, K_b the basic dissociation constant, and K_w the dissociation constant of water. K_b has been found to be 4.8×10^{-12} for gelatin, hence $K_a = 3.5 \times 10^{-7}$. Miss Jordan-Lloyd has investigated the acid properties of gelatin and finds a series of steps corresponding to the coming into action of various groups. But it seems that to explain completely all the experimental facts it is necessary to assume various kinds of combination with the peptide linkage and changes in constitution by the action of the sodium hydroxide used in titration. Miss Jordan-Lloyd is not satisfied with the value of the acidic dissociation constant

as calculated above, but if the effective molecular weight as a monobasic acid is taken as 839, the carbonic acid displacement values to be referred to below correspond fairly well with the calculated value of the dissociation constant.

That other ions than those of hydrogen have to be taken into account is shown by the fact that Loeb found a positive charge given to "isoelectric" gelatin by cerium salts. It has been noted above that multivalent ions are especially powerful in reversing electric charges. A difficulty with regard to the indiscriminate use of the conception of isoelectric points as referring only to the hydrogen-ion and to amphoteric electrolytes is met with in cellulose. The negative charge was found by Perrin not to be completely reversed by hydrochloric acid of $\frac{m}{20}$ strength. If this is to be interpreted on the basis of the ratio of the two dissociation constants of an amphoteric electrolyte, cellulose must be assumed to have strongly acid properties.

More objection must be made to the

application of the conception to the case of blood corpuscles, as has been done. Here the surface charge is due to the layer of anions arising from the impermeability of the membrane for cations and its permeability for anions. "Isoelectric point" in this case means simply that concentration of acid which balances these anions, and any other cations would serve the same purpose.

The various ways in which a surface may obtain an electrical charge have been mentioned above and all these possibilities must be kept in mind.

Adsorption of Ions

That a charge may be given otherwise than by electrolytic dissociation of the surface is shown by the existence of negative ferric hydroxide, as present in alkaline solution. This is not a sodium salt of the nature of a ferrate or ferrite, as is shown by the fact that although it does not diffuse

through parchment-paper, it has no osmotic pressure, as a sodium salt would have. There seems to be no other way to explain the sign of the charge than that which attributes it to the adsorption of hydroxyl-ions.

As regards proteins, we have seen that they do not combine with either acids or bases at their isoelectric points. When therefore they take up neutral salts, these must be adsorbed. In the matter of precipitation, Holker finds that the opacity produced by sodium chloride in albumin solutions follows the curve of an adsorption process.

On the other hand, Pfeiffer and Modelski described what they looked upon as true chemical compounds of glycine and alanine with chlorides of potassium and calcium. If this happens with amino-acids, it is possible that it might happen with proteins also. On repeating the experiments of these workers, I was myself unable to obtain evidence of definite compounds. But Harold King finds that under certain

conditions it is possible to get what may be regarded as a kind of compound, in which there is a molecular relationship, the inorganic salt being attached in a way similar to that of water of crystallization. This is, of course, not the same thing as the combination assumed by Pfeiffer and Modelski. No evidence has yet been found that proteins form compounds of this nature.

The Nature of Proteins

If we look at the curves correlating the properties of protein solutions with their hydrogen-ion concentration, we note that the isoelectric "point" is a fairly wide region. In the curve of imbibition by gelatin given by Miss Jordan-Lloyd, there is practically no change between hydrogen-ion concentrations of 10^{-4} and 10^{-9} . In this region proteins can be obtained which have no higher electrical conductivity than that of water. Thus, they are not acids or bases in the state in question. Hydrogen-ions

and hydroxyl-ions in fact cannot exist together in equal numbers except in the very minute concentrations in which they are present in water.

In order to account for the inert behaviour of proteins uncombined with acid or base, Gustav Mann made the suggestion that the carboxyl- and amino-groups were not free, but combined together in a ring form as an internal ammonium salt. In order to split this ring, a certain minimum strength of acid or alkali may be required, somewhere about 10^{-4} or 10^{-9} in hydrogen-ion concentration respectively. This view is quite in accordance with the facts that within these limits serum proteins have no perceptible effect on the reaction of weak acid or alkali. But perhaps the phenomenon is only an aspect of the very flat form of the curves about the isoelectric region. Practically, of course, the results are the same.

It appeared to me that one might obtain some idea of the acidity of proteins by estimating their capacity of displacing

carbon dioxide from a solution of sodium bicarbonate. That is, the relative amount of the acid in question and of carbonic acid in combination with the sodium. While that of acids in true solution followed closely, in equimolecular concentration, their accepted acidic dissociation constants, as shown by the table below, proteins did

Acid.	Dissociation constant $\times 10^7$	Theoretical CO ₂ displaced.	Found.
Aspartic ..	1500	99.8	97.3
Lactic	1380	99.8	100
Benzoic.. ..	600	99.5	94
Uric	15	83	71.6
(Carbonic ..	3.04	50)	
Alloxan.. ..	2.3	43	39.9
p. Nitrophenol	0.7	18.7	38.8
Glycyl-glycine	0.18	5.6	10.4
Tyrosine ..	0.04	1.3	0

not appear to be as strongly acidic as would be expected. But one must not attach too much value to the data with regard to proteins, owing to the uncertainty as to their molecular weights.

Carbonic acid itself is inserted in order to show its position in the series.

An interesting point is that casein when rubbed with a solution of potassium phosphate with a hydrogen-ion concentration of 10^{-7} , considerably more alkaline than its isoelectric point, does not form potassium caseinate.

Sørensen found that the osmotic pressure of solutions of egg-white as progressive amounts of sodium hydroxide were added rose in a very steep curve, whereas on the addition of acid very little change occurred for some distance from the isoelectric point. Even on comparatively large additions, the rise was much less than on the alkaline side. I have myself found similar facts, which suggest that there are some factors concerned not as yet understood.

The ultra-filtration experiments of Cushny show that no sodium is combined with protein in blood serum. The same conclusion follows from the measurements made by Neuhausen with a sodium electrode ; all the sodium is in ionic state. This same observer also finds that the vapour pressure of serum corresponds to that

of its sodium constituents. On the other hand, it appears that a part of the calcium content is not in the ionized form. Although this has been taken to mean the presence of a compound with protein, it seems more probable, from the results of Brinkman and van Dam, that the undiffusible, non-ionized calcium was in the form of calcium carbonate in a colloidal form. No special precautions were taken, in the experiments referred to, to keep the carbon dioxide content the same as in blood.

Imbibition

As is well known, colloids are usually divided into "suspensoids" and "emulsoids," the former consisting of the solids which are free from water, such as gold, arsenious sulphide and so on. What should be understood by the latter is not quite so clear. They are sometimes defined as those colloidal systems whose dispersed phase is liquid, or more or less so. But there are

two different kinds of systems of this nature, with different properties. In the one, the dispersed phase is a liquid immiscible with water, and as a rule free from water. An emulsion of paraffin oil may serve as an example. Such systems are more like suspensoids in their behaviour, whereas the other class, of which the proteins are typical, consist of more or less liquid particles, but made so by the fact that they contain a fairly large proportion of water. It will be clear that the two phases in such systems differ from each other in composition much less than in the two previous classes, and that the properties at the interface, such as surface tension, electrical charge and so on will be less pronounced. It would be more useful if the term "suspensoid" were allowed to include emulsions of anhydrous liquids and if the term "emulsoid" were limited to those cases in which the more solid phase contains water, so that the two phases merely differ in the relative proportion of water which they contain. An emulsoid would then be

defined as a two-phase system, in which the more solid phase differs from the liquid one in containing less water, both consisting of water plus solid. I admit that such a definition would exclude "emulsions" of fat, which is certainly an objection to it. But this would be outweighed by the advantages. Otherwise, a new name for such systems as the proteins would be necessary. The names "lyophile" and "lyophobic" do not seem to have been generally accepted, although they give the correct interpretation.

It has been pointed out by Hatschek and by Freundlich that the distribution of water between the two phases of an emulsoid can be varied by certain agents, more especially by electrolytes. This is clearly a very important property in connexion with the dynamics of the cell, as will be apparent when we come to the discussion of enzymes.

There appear to be two ways in which this happens, although the second of these is denied by some investigators. Loeb

and Proctor hold that the process is essentially one of osmosis, and perhaps the best way to understand the points at issue is to consider first an old experiment made by Hardy. If a solution of five or ten per cent. gelatin in warm water is allowed to cool, the well-known jelly is formed. This jelly, although it contains ninety per cent. or more of water, maintains a shape into which it is cut and has a certain amount of elasticity in that if deformed it returns to its original form when released. For our present purpose, its most interesting property is that water cannot be squeezed out of it merely by pressure with the hand ; a very high pressure is required to do so. Suppose now that we allow a lump of it to lie in a five per cent. solution of formaldehyde. It will be found that, after a time, water can be pressed out of it by the hand. There must be two different kinds of structure in the two cases. In the first, the water phase, really a dilute solution of gelatin, must be imprisoned by walls of the more solid phase, like cells of a honey-

comb, so that if the liquid is to escape it must be filtered through the solid. In the second case, it is clear that the watery solution, as well as the more solid phase, must be continuous. This would happen if holes were formed in the walls of the honeycomb cells. Both phases are in fact continuous. It is naturally difficult to make out these different structures, even by the ultra-microscope, on account of their minute dimensions, so that it cannot be regarded as certain that they are precisely as stated.

The next step is to imagine what takes place when such a structure as the first of the two described, namely the honeycomb, is put into a dilute alkaline solution. The gelatin swells by taking up more water. How does this happen? The gelatin of the watery phase contained in the closed cells, by combination with the diffusible alkali, rises notably in osmotic pressure: the protein salt being indiffusible attracts water through the walls and distends the cells. But there is more than this. Gelatin

in which it is improbable that a honeycomb structure is present is capable of swelling in alkali. There is nevertheless a solid phase and, according to the theory of Proctor, this solid phase may itself act osmotically in attracting water. We saw in our consideration of the Congo-red system that owing to the impossibility of the dye passing out through the membrane, all its ions are held back. It is possible to imagine that if instead of being restrained by a membrane, these ions were held by the fixed solid structure of the gelatin phase, this phase would exercise an osmotic effect in attracting water to itself. The precise situation of this water as regards the elements of the structure is not a material question. It looks rather as if it must be supposed to be on their surfaces.

We may further apply, as Loeb does, Donnan's theory of membrane equilibrium to the action of neutral salts. In presence of such salts, gelatin swells less than in their absence. We have seen how this theory explains the lowering of the effective

osmotic pressure of a colloidal salt. It therefore fits in well with the osmotic view of the swelling of gelatin. But of course it does not come into play when foreign electrolytes are absent, although Prof. Loeb in some statements seems to suggest that it does.

There are certain facts which indicate that there are some other factors involved in the imbibition of water by colloids. There is indirect evidence that the taking up of water by imbibition is, at all events in part, a process of adsorption. Posnyak in his work on imbibition by gelatin finds that a formula with a fractional index similar to that of adsorption satisfies his results, although he thinks that solid solution is also involved. Colloidal copper ferrocyanide takes up different amounts of water, as it appears, according to the varying extent of surface. An observation that I made myself on the taking up of water by dry gelatin from ninety per cent. alcohol is difficult to explain otherwise than as a surface adsorption. The alcohol was found

to have lost water, but the gelatin immediately after removal from the liquid and drying of its surface by filter paper had not increased in weight. It is possible that evaporation of the alcohol might carry off surface water, but it seems scarcely possible that "imbibed" water should escape so rapidly from the interior of the gelatin sheets.

We have next to consider some facts in reference to the series of anions known as "Hofmeister's series." Prof. Loeb has recently cast doubt on the existence of any difference between the effects of univalent anions on the swelling of colloids, as had been described by previous workers. This he does on the ground that this difference vanishes when the hydrogen-ion concentration is kept constant. It is certainly desirable that such experiments should be made under more carefully controlled conditions, but the effects seem to be too great to be due merely to the slight differences in hydrogen-ion concentration, while there are facts which indicate the existence of an effect on the distribution of water between the

two phases due to an action on the compressibility of water, apart from the formation of definite salts in stoichiometrical ratio. When a colloidal substance swells by taking up water, it is found that the total volume of the system diminishes to some extent. This can only be explained by a compression of water. In cases where the magnitude of the effect has been measured, it is found that a pressure of some 400 atmospheres must have been brought into play. That surface molecular forces are quite sufficient for the purpose is shown by the calculations of A. N. Williams, who finds that the attractive (cohesive) pressure in the surface film on charcoal is of the order of 10,000 atmospheres. He points out that such compressive forces by their effect on electrolytic dissociation may give rise to a higher concentration of the ions of water in an adsorbed layer than in the body of the liquid and thus to a diffusion potential which may play a part in the surface charge. One may note incidentally that heat is liberated in the process of imbibition by

emulsoids and has been measured in some cases. Now if we take the series of salts with different anions comprised in the Hofmeister series, we find that their relative effect on swelling is in the order of their action on certain properties of water expressed in compressibility, surface tension, and so on. Schryver showed that a similar state of affairs is present in the dispersion of colloids such as globulin, as also in the displacement of the temperature at which a mixture of phenol and water becomes homogeneous (the "critical solution temperature"). Difference in degree of adsorption of these various anions may well have a notable effect on the properties of water at the interface and lead to modifications in the amount of water present in the more solid phase, as pointed out by Hatschek and by Freundlich.

But whatever may be the mechanism, the important fact is that the capacity of taking up water reversibly by the colloids of the cell may be regulated by such agencies as free electrolytes.

Although acids and alkalis have so powerful an effect on the swelling of colloids, it is to be remembered that a fairly high concentration is necessary and that the presence of neutral salts is inimical to it. The theory of œdema put forward by Martin Fischer is thus inadequate. Apart from the incapacity of such small changes in acidity as are possible in the blood to produce other than minute degrees of swelling, there are other grounds, such as the existence of free liquid in the tissue spaces, which show that œdema is really a phenomenon of osmosis.

Viscosity

The measurement of the viscosity of colloidal solutions is a useful method of investigating changes in their state. Moreover, if there were a simple accurate method of measuring the viscosity of blood, it would be a valuable clinical means of estimating the changes in the number of corpuscles,

or concentration of the blood. A few words with reference to the principles involved may be of interest.

A formula was put forward by Einstein as applying to a suspension of rigid spheres not too close together to interfere with each other's movements. If η is the viscosity of the liquid medium of suspension, f the ratio of the volume of the suspended phase to that of the medium, and k a constant, then the viscosity of the system (η_r) is given by the formula :

$$\eta_r = \eta (1 + kf).$$

In the case of various suspensoids the value of the constant varies from 1 to 4.75. The increase of the constant has been supposed to be due to departure from sphericity, but Jeffery shows that this would cause a fall in the constant. An important implication of this formula is that the mere aggregation of the particles would not affect the viscosity. But if water passes into or out of the suspended phase, then the value of f

changes, owing to what amounts to an increase or decrease of concentration of the suspended phase. The same statement applies to the logarithmic formula preferred by Arrhenius :—

$$\log \eta = \theta c,$$

where θ is a constant and c the percentage volume of the system occupied by the particles. The viscosity of water is taken as unity. Bazett found this formula to apply satisfactorily to blood as diluted with plasma, if θ be given the value 0.00773, the total viscosity being given by multiplying by that of the plasma.

If the suspended phase is deformable, as in the case of emulsoids, Hatschek finds the following formula to hold :—

$$\eta = \frac{\sqrt[3]{A}}{\sqrt[3]{A} - 1}$$

where A is $\frac{\text{vol. of whole}}{\text{vol. of dispersed phase}}$.

Thus the total volume of the dispersed phase in relation to that of the dispersion medium is the determining factor. Degree of dispersion does not affect the viscosity, but changes in the hydration of the particles do.

This being so, we should expect very little change in the viscosity of blood when the corpuscles are hæmolyzed. This is the case. The rise of viscosity produced by saponin is due to some secondary effect on the stromata. It has been found by my son, L. E. Bayliss, that sufficient saponin can be added to break up the corpuscles without any significant effect on the viscosity. The addition of further quantities, however, results in a large increase.

CHAPTER V

HÆMOGLOBIN

Difficulties

THE preceding discussion of colloids and especially that of proteins naturally leads to some remarks on hæmoglobin. It is a remarkable fact that this pigment together with chlorophyll, chemically allied, are the two most interesting and at the same time most puzzling of all known substances. We may also venture to say that they are also the most important, since on them depends our own supply not only of food but also of the oxygen to burn it. They have moreover their æsthetic significance. It will scarcely be denied that the two things that give us

most pleasure are rosy cheeks and green fields.

What makes hæmoglobin especially difficult to investigate is the combination of remarkable chemical and physical properties, which are unknown as yet in any other compound. Although it has been the subject of research for so many years, some of the most fundamental problems remain still unsolved. It is perhaps in this case that the apology which I felt bound to make in the Preface to this book is especially needed. Because the work done by Barcroft and his school, by A. V. Hill and by Haldane is of so much value as regards the actual data obtained, which are permanent and must serve as basis for future work, any criticism that I make must be understood to refer rather to the validity of certain interpretations or to the extension of particular cases to general laws. More especially does it seem necessary to speak a word of caution against the assumption that the current hypothetical interpretation of the constants of the Hill equation

suffices to explain all the phenomena. If any remarks made serve to suggest problems for experimental work, they will have had their use.

Relation to Oxygen and Other Gases

The equation found by A. V. Hill to satisfy the experimental data of Barcroft is :

$$\frac{y}{100} = \frac{Kx^n}{1 + Kx^n}$$

where y is the percentage saturation, x the oxygen pressure, K and n appropriate constants.

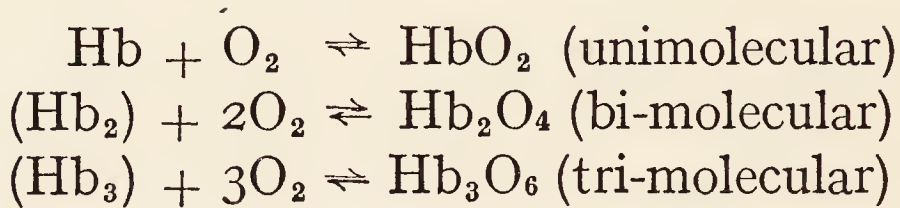
Although this was at first put forward merely as an empirical formula without any physical meaning attached to the constants, it was not long before n was interpreted as the order of a reversible reaction on mass action basis and K its equilibrium constant.

It may be said in general that the experi-

mental data do not fit very exactly in any curve, or, alternatively, more than one curve may be made to fit equally well. Haldane's equation is slightly different from Hill's. An equation with two constants, however, is of a somewhat elastic nature. What is intended to be conveyed by these remarks is that the fact of the oxygen-dissociation curve being expressed by Hill's formula, or any similar one, does not prove much.

At the isoelectric point for hydrogenions and in the absence of salts, $n = 1$, so that the curve becomes the rectangular hyperbola of a unimolecular reaction. But in the presence of salts, carbon dioxide, etc., of the blood, $n = 2.4$, that is, the reaction as a whole is between a bi- and trimolecular reaction. To explain this, it has been suggested that hæmoglobin may exist in the blood in the form of aggregates of two, three or more molecules. Since each of these as a unit would combine with two, three or more molecules of oxygen respectively, the reactions might be re-

garded as being bi- or tri-molecular and so on. Thus :



and so on.

If now we take the general equation and suppose appropriate relative proportions of different integer values of n , we can arrive at any wished for mean fractional value. Thus, if we take two parts of one, one part of two, and one part of three, the mean is 2.33. But I am told by a mathematical friend that this is not permissible. We must take each separately as a whole in different proportions and then evaluate the mean. If we do this, we obtain complicated algebraical expressions which imply a mass action relationship between the concentrations of the different aggregates, together with additional constants, by which any imaginable curve could be traced. Apart from such procedure, the results appear to be indeterminate.

Since hæmoglobin is an emulsoid, it is not to be expected that univalent ions, such as those of sodium chloride, would have much effect in causing aggregation. Adair was unable to find evidence of such aggregation by osmotic pressure measurements.

Moreover, since the H-ion isoelectric point is somewhat on the acid side of the reaction of blood, hæmoglobin should, at all events in part, be present as a sodium salt. This could doubtless be tested by ultra-filtration, but has not been done as yet. But if it is the case, the hæmoglobin ions are probably aggregated to a much greater degree than the equation assumes. The question arises again in regard to the osmotic pressure measurements of Hüfner and Gansser. If their hæmoglobin was partly a sodium salt, it must have been dissociated and, unless it behaved as a dibasic acid like Congo-red, it is remarkable that the value found corresponded exactly to its supposed molecular weight on the basis of its content in iron. In the free state at

the isoelectric point recent observers find very low values of osmotic pressure, like other proteins. There is obviously something that needs explanation here.

But, after all, the suspicion cannot help arising as to whether oxyhæmoglobin is a true chemical compound. The fact that the amount of oxygen taken up in saturation corresponds to the content in iron undoubtedly suggests that it is. But then we are met with the existence of methæmoglobin, which is undoubtedly a chemical compound with oxygen, since the oxygen can only be removed by a chemical reducing agent, not by a mere vacuum for oxygen. This methæmoglobin would repay further investigation. It is easily formed in slightly acid reaction, and perhaps free oxyhæmoglobin may only exist with difficulty, so that what we call oxyhæmoglobin is the sodium salt. On the other hand, there seems to be very little evidence that hæmoglobin is combined with sodium at all at the hydrogen-ion concentration possible in blood. But this involves other problems with

respect to the carriage of carbon dioxide. Further problems to be worked out!

Without proposing to carry the point further, I cannot avoid reminding the reader that a fractional index is a characteristic of most adsorption processes. There is indeed a curious similarity between the form of Hill's equation and that given by Langmuir for the adsorption of a gas by a crystal surface :

$$m = \frac{\alpha \mu}{1 + \alpha \mu}$$

where m is the amount of gas taken up, μ is a function of the gas pressure and α is a constant rather less than unity.

A few words are necessary as to theories of the carriage of carbon dioxide. It seems fairly certain that this takes place by the agency of hæmoglobin, and most observers hold that it is due to a competition between hæmoglobin and carbon dioxide for the possession of available sodium. This proportion of sodium combined with each would naturally vary with the tension of

carbon dioxide. But if we accept the value given to the acidic dissociation constant of hæmoglobin, it can be calculated that only a small fraction of the sodium is combined at any time with hæmoglobin, about 5 per cent., the rest being present as bicarbonate. Further, recent experiments show that the contents of the red corpuscles are more acid than the plasma, and this would imply even less sodium in combination with hæmoglobin. It is to be admitted that the above calculation rests on uncertain assumptions as to the effective molecular weight and concentration of hæmoglobin, while the mode of deducing the acidic dissociation constant may not be correct where colloids are concerned. Oxyhæmoglobin is further stated to be a much stronger acid than reduced hæmoglobin and thus capable of driving out carbon dioxide from bicarbonate in the lungs. But the chief evidence rests on measurements with the hydrogen-electrode and the differences found are within the errors said by Sørensen to be inherent in

the method when proteins are present. At any rate, the mechanism on this basis does not appear to be such an effective one as so important a function would lead us to expect.

Numbers of more or less exact clinical curves have been constructed on the basis of mass action formulæ as applied to the acid-base equilibrium in the blood. That these cannot all be reliable is shown by the discovery of Lovatt Evans that lactic acid is rapidly formed in shed blood by glycolysis. To avoid this, about 0.1 per cent. of sodium fluoride must be present.

It has been held by some physiologists that hæmoglobin itself takes up carbon dioxide in a way similar to that in which it carries oxygen. Bohr, several years ago, published a dissociation curve of what he believed to be carbon-dioxide-hæmoglobin, free from sodium. It is to be remembered, however, that if two atoms of sodium combine with one molecule of hæmoglobin, there would be only one part by weight in 350, probably escaping detection by

chemical analysis. Some similar results have been obtained by Buckmaster, but the problem is not decided. Mrs. Wright Wilson has observed that carbon dioxide causes a large rise in the osmotic pressure of hæmoglobin. I have myself found that blood dialysed against 1 per cent. boric acid ($pH = 4.7$) gave off, after exposure to alveolar air, more carbon dioxide on acidification than was present in the bicarbonate also added. Presumably, there was no sodium salt of hæmoglobin at this hydrogen-ion concentration.

It seems to the writer that some progress might be made from the point of view of heterogeneity and surface properties, by way of variety, instead of on the a priori assumption that the system obeys the simple mass action laws of a homogeneous one. At least, the possibility might be kept in mind. Even if it proved wrong, something would be gained. Certain facts suggest that adsorption is an important factor, but that the peculiar nature of the compound introduces complications. Thus

there may well be chemical combination regulated as to degree by adsorption. Nevertheless, it would be an attractive result if it turned out that oxygen, carbon dioxide, carbon monoxide, nitric oxide, and chloroform were taken up by a similar process. On the face of it, it would seem rather unlikely that hæmoglobin should have chemical affinity for all of these dissimilar substances. But it remains to be seen. All that can safely be said is that adsorption has not been disproved.

The Phase Rule and Colloids

Oxyhæmoglobin as a chemical compound appears to be unique in its property of containing various quantities of oxygen in the presence of an unlimited phase of oxygen at different pressures. Other chemical compounds which take up oxygen, so far as known, behave like the heterogeneous system of lime and carbon dioxide; that is, they are either saturated with or free

from oxygen. In the language of the phase rule, these latter have but one degree of freedom, whereas hæmoglobin has two. In other words, if hæmoglobin is a heterogeneous system, it does not obey the phase rule as a chemical compound should.

But the difficult question arises here—Does this rule apply to colloidal solutions? It will be remembered that Gibbs excluded all factors except pressure, temperature, and volume. Minute colloidal particles, although certainly a separate phase, may be peculiar in that such factors as surface energy and electric charge may interfere. The matter turns rather on what we are to understand by solubility. It may be noted that Parsons in a recent paper assumes periodic precipitation of hæmoglobin at various stages of acidity.

Hence, it would be of interest to make experiments in which hæmoglobin is present as an undoubted phase in the sense of Gibbs, say in moist films on glass or as deposit in a saturated solution, in

order to see if it behaves in the same way as in ordinary solution—in equilibrium at the same temperature with various tensions of oxygen forming a regular curve, not all hæmoglobin at one pressure and all oxyhæmoglobin at a slightly higher one, like the calcium-oxide carbon-dioxide system. The fact that oxyhæmoglobin crystallizes out when concentrated solutions of reduced hæmoglobin are shaken with oxygen or with carbon dioxide may be useful for this purpose.

Heat of Combination

Hæmoglobin in taking up oxygen gives off heat. Barcroft and Hill measured this and found that it corresponded to the theoretical value for a chemical compound. Subsequent workers obtained very divergent values, for the most part lower than those of the workers mentioned. The latest results are those of Adolph and Henderson, who used a method accurate to 1 per

cent. They found values varying enormously. They can only suggest that there are some unknown factors as yet uncontrolled.

CHAPTER VI

ENZYMES

Heterogeneous Catalysis

THE facts that enzymes are in the colloidal state and that their action takes place on the surfaces of the enzyme phase are now generally admitted. Numerous cases are known where this phase is in the form of coarse insoluble matter that can easily be filtered off, the filtrate being then inactive. An experiment of this kind can easily be made with urease. If powdered soy-beans are shaken with 70 per cent. alcohol and the suspension then filtered through paper, it will be found by adding urea that no enzyme is in solution in the filtrate, but that the

powder on the filter is very active on a solution of urea in 70 per cent. alcohol.

We have to do accordingly with a special case of heterogeneous catalysis and we may proceed to consider some properties of enzymes in which surface action plays a part.

Relation to Adsorption

It will be clear that since the action takes place on the surface, adsorption of the interacting substances, say water and ethyl butyrate, must be the first stage. And further, that the rate of reaction should be proportional to the amount of these substances adsorbed at a given moment. It follows from this that the extent of surface available is a controlling factor and we may profitably examine into what conditions regulate it.

It is well known that certain substances which are powerfully adsorbed, such as saponin, urethane, the higher alcohols and so on, retard the action of most enzymes

to a greater or less extent. The alcohols have a mixed effect to be referred to later. That of saponin is less complex. Since adsorption is less at higher than at lower temperatures, it occurred to me that if saponin retards by obtaining partial possession of the surface of an enzyme, its effect should be greater at the lower temperature. This was found to be so. It was also noted that the action of saponin does not permanently injure the enzyme, since in the end the same effect is produced as in its absence. This retarding action of saponin is almost prevented by charcoal, because saponin is adsorbed by charcoal to a greater extent than it is by the enzyme.

Northrop holds that in the case of trypsin, adsorption of the protein substrate by the enzyme cannot be a controlling factor, because when two proteins are present together, the effect is the sum of the effects on both, whereas one would have expected that the adsorption of one would leave less surface for the other. The conditions are more complex, however, in the case

of proteoclastic enzymes than in those where the substrate is in true solution. We have two colloidal phases, and it is not quite clear as to which should be regarded as the adsorbing surface. Probably experiments with two substrates and varying enzyme concentration might throw light on the problem. There is a further possibility which does not seem to have been investigated. Suppose that a film of casein has been adsorbed, say by charcoal, it is not unlikely that this casein surface may in its turn adsorb another substance, perhaps albumin. Experiments to find out whether an adsorbed layer behaves like the surface of a mass of this same material would give interesting information. We have seen that a surface may become saturated—on Langmuir's theory, when covered by a layer of one molecule in thickness. It has been suggested that this is the reason why above a certain concentration of substrate, no further increase of rate of change occurs. But it is stated by Northrop that in the case of

trypsin, if the course of the reaction be followed by estimation of the amount of protein left unchanged, this phenomenon does not appear. It is difficult to see how this applies to urease and lactase, where the chemical change is comparatively simple, but it should be tested.

*Colloidal Properties and Consequences
Therefrom*

The active surface may be diminished by aggregation of a number of small particles to form one large one, as well as by its being partly occupied by a foreign substance in the way discussed above.

Enzymes, however, are not particularly sensitive to electrolytes, except to hydrogen- and hydroxyl-ions. This is probably because they are emulsoids, or at any rate complexes of which emulsoids form a part. The trivalent lanthanum-ion in point of fact precipitates urease, completely in 0.005 molar concentration, aggregates the enzyme

in 0.00083 molar solution. In the latter case, the activity is reduced to four-ninths of its normal value. Tadokoro showed by the spectro-photometer that calcium-ions aggregate taka-diastrase. Onodera investigated the effect of a number of acids on urease and came to the conclusion that their surface tension plays a part, perhaps by facilitating adsorption of the hydrogen-ion component.

Since electrolytes alter the distribution of water between the two phases of an emulsoid system, an investigation of the results of varying degrees of distension of enzyme particles by water would be of interest.

The opposite effect of greater dispersion in increasing the active surface is sometimes met with in the results of the addition of substances which lower surface tension. Bile salts increase the activity of lipase, capryl alcohol that of urease. The lowering of surface tension by the adsorption of these substances causes greater dispersion because it allows smaller particles to exist

without joining together for the purpose of reducing their surface energy.

It will be noted that this property of "surface-active" substances is in conflict with that of possession of surface in reducing the amount of substrate adsorbed. The opposition shows itself in the action of alcohols, whose effects differ according to concentration. A further complication is also present here in that they have also a destructive effect, due either to chemical action or more probably to irreversible aggregation. If we compare the effects of capryl, amyl and ethyl alcohols on urease, we find that the first is purely favourable, on account of the fact that its effect on surface tension is great, while its solubility is too low for much adsorption and displacement of urea from the surface. The effect of amyl alcohol is like that of saponin, the displacement effect is preponderant. Ethyl alcohol retards by aggregation; it has very little influence on surface tension. Onodera finds that it accelerates in weak concentration.

Enzymes when precipitated by alcohol or acetone can be redispersed in active form if not kept too long exposed to the reagent. In such a case, the irreversible form of aggregation described by Chick and Martin (p. 69 above) occurs.

It is not unlikely that the high temperature coefficient of enzyme-action may be partly due to greater dispersion, which has been seen in the ultra-microscope as a result of moderate warming. On the other hand, inactivation at higher temperatures may be due to irreversible aggregation; probably the precipitate is not the active constituent of the enzyme system, but carries the latter down by adsorption. An enzyme adsorbed on charcoal or when substrate is adsorbed on it is less sensitive to heat. The particles in such circumstances are unable to aggregate.

Theories of Mode of Action

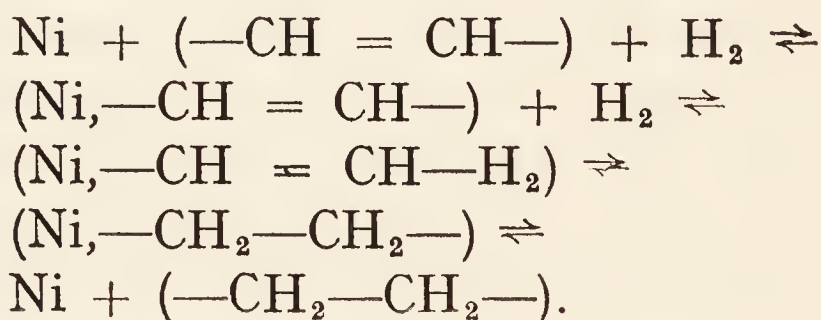
A word may first be said as to the value

of applying mass action formulæ. The fact, now generally accepted, that enzymes act at their surfaces, introduces a difficulty at the outset. Although a unimolecular formula is occasionally found to apply fairly well to a particular stage of an enzyme reaction, the fact that the rate of further change is proportional to the amount then left unchanged is by no means a peculiarity of a simple chemical reaction, and indeed it gives little if any information as to the nature of the mechanism concerned. When dealing with colloids, it is only in a high state of dispersion that the surface varies even approximately as the mass concentration. As the size of the aggregates increases, the disproportion becomes greater. It is very doubtful whether progress is possible on the simple lines of mass action.

On the other hand, it is by no means clear how surface concentration leads to increased rate of reaction. If we accept Langmuir's point of view, the reacting groups of the adsorbed molecules may be so orientated as to be favourably situated for mutual

interaction, or the reverse (as in the experiments of van Kruyt). This might be called a static view. It has been pointed out that it excludes mass action and also theories of intermediate compounds with the enzyme itself.

Although theories of this latter kind enjoy some reputation, the general occurrence of such intermediate compounds in heterogeneous catalysis is purely hypothetical, unless we choose to look upon adsorption as the formation of an intermediate compound. As an example of such theories, we may take the series of equations suggested by E. F. Armstrong for the hydrogenation of unsaturated fats under the influence of a nickel catalyst. The symbol $\text{—CH} = \text{CH—}$ represents the ethylene linkage of a fat :



One may ask, does this really help or mean more than if the nickel had been left out?

Hardy has suggested that the chemical potential of reacting groups may be raised by surface forces, and in this connexion we may remember the calculations of Williams as to the great magnitude of such forces (see p. 96 above). Langmuir also found that hydrogen becomes atomic on tungsten filaments.

Faraday was apparently of the opinion that close approximation on the surface is sufficient to account for the union of oxygen and hydrogen on platinum and other inert surfaces.

If we take the view that the increased rate of reaction is due to the mass action of increased concentration, it must be admitted that after fixation by adsorption the usual kinetic effect of greater frequency of collision cannot come into play. But may it not be that the acceleration occurs in the liquid phase, in the layer immediately preceding adsorption? The concentration

in this layer is naturally brought about by the forces of the interface.

When we have to do with a reversible reaction which has its equilibrium position some distance from completion in either direction, such as the hydrolysis of an ester in the presence of very little water, an interesting question arises which as yet awaits an answer. As is well known, the equilibrium position is arrived at in the absence of a catalyst, if we wait long enough. It can be brought about rapidly by the catalytic agency of an acid and is found to be at the same place as the natural one, since it is characteristic of such homogeneous catalysts that they do not supply energy to the system. When catalysis takes place on the surface of a heterogeneous phase, as by an enzyme, it seems improbable that all the constituents of the system should be adsorbed in the same proportion as they exist in the liquid phase. Some of them would no doubt be adsorbed more strongly than others. If this is the case, the equilibrium position would be

different on the enzyme surface from the natural one in the body of the liquid phase. Does this imply that the equilibrium in catalysis by enzymes is not the same as that brought about by acids? Dietz did actually find that the position in an ester system was nearer to the ester side, or synthetic position, when brought about by lipase than when brought about by acid. This apparently puzzling result implies that energy was supplied from some source. Dietz suggests surface energy. Supposing that water was less adsorbed than the other constituents of the ester system, as is probable, the reaction towards the synthetic side would be favoured, with the supply of energy from surface action, perhaps heat of adsorption. Some information as to change of equilibrium position by differential adsorption might be obtained from experiments in an ester system in presence of a large adsorbing surface, say charcoal. I have such experiments in progress at the present time.

Synthetic Action

The facts mentioned in the preceding paragraph lead to brief reference to the synthetic activity of enzymes. It is sufficient to recall the fact that the equilibrium position in a reversible hydrolytic reaction depends on the concentration of water in the system to see that the same enzyme will have either hydrolytic or synthetic action according to circumstances. There is no necessity for the assumption of special synthesizing enzymes, and indeed an examination which I made of the cases in which they were supposed to exist showed the falsity of the hypothesis.

Adjustment of Processes in the Cell

We see therefore that what is wanted to control the sign of the activity of enzymes in the living cell is an effective method of changing the concentration of free water. For example, the amylase of the liver at

one time may have the function of hydrolysing the stored glycogen ; at another, the synthesis of this glycogen by removal of water from glucose molecules.

One way of doing this is by the action of electrolytes on emulsoids, changing their state of swelling. Or water may be adsorbed on certain surfaces, the area of which may be increased or diminished by various agents, as that of colloids, by dispersing or aggregating agents.

CHAPTER VII

THE CELL-MEMBRANE

WE turn now to the part played by adsorption in the properties of the membrane covering the surface of living cells, which is of so much importance in the regulation of the permeability, osmotic pressure and related characters of the cell.

A significant fact was discovered by Pfeffer, Naegeli and others, when they found that a new film, of the same properties as the original one, was formed when the protoplasm of the cells was broken up so that the surface of the fragments came into contact with water.

Mode of Production

It is evident that the film must be

produced by the surface condensation of some constituents of the cell protoplasm, those constituents in fact which have the property of reducing surface energy of some kind.

Further, some of these constituents when concentrated at an interface become more or less rigid, as shown by Ramsden. It appears that the limit of solubility or of colloidal suspension is exceeded by such concentration. Substances which behave in this way are proteins, peptone, bile salts, quinine, saponin, etc. When the outside liquid phase itself contains substances capable of surface condensation, as in the animal organism, these substances themselves take part in the formation of the membrane.

Properties

An interesting result of the mode of formation of the membrane is the possibility of the passage through it of solid particles of visible dimensions, although in ordinary

circumstances it is impermeable to colloids and even to certain inorganic salts, such as sodium chloride. This is due to the actual breaking of the membrane by the particles and its subsequent new formation behind them as they pass through. A needle may be passed through a soap film in the same way. We have here the explanation of phagocytosis. Substances in the ionic or colloidal state must pass through pores that are already present, and if these are too small, the membrane is impermeable for such substances.

It is a matter of great difficulty to suggest any probable structure for the membrane. Owing to its mode of production, it is no doubt of a very complex chemical nature. It appears to be, in all states of the cell, permeable to all substances soluble both in water and in oil or lipoid, such as urea, some ammonium salts, alcohol, chloroform, carbon dioxide, oxygen, etc., as Meyer and Overton pointed out. In the resting state of the cell, the membrane is impermeable to salts, other than certain salts of

ammonium, to glucose and to amino-acids ; while in a state of activity it becomes permeable to all these. The facts mentioned support the suggestion made by Clowes that the membrane is a system of two phases, a watery solution of protein and a lipoid phase. In the resting state, the lipoid phase is the external or continuous one, so that the pores between the elements of the watery phase are filled with lipoid and can only be passed by substances soluble therein. In the state of activity, the position of the two phases is reversed. Hence the watery phase is now continuous, so that any substance soluble in water, even if insoluble in lipoid, can now pass through. As already referred to (p. 26 above), Clowes showed that artificial systems of the nature in question could be converted into one another. The oil-in-water emulsion is converted by calcium salts into the water-in-oil system. The effect of calcium in maintaining the normal state of the cell is significant in this connexion and will be considered again later.

According to such a view of the structure of the cell-membrane, there are two conditions necessary for a substance to penetrate it. This substance must be soluble in what happens to be the continuous phase and also of sufficiently small molecular or particulate dimensions to pass through the pores between the elements of the dispersed phase. In considering molecular or ionic dimensions, the associated water molecules must be taken into account. Hamburger has suggested that the shape of the pores in relation to that of the molecules traversing them may play a part.

This impermeability of the living cell-membrane for most solutes has important results. It is clear, to begin with, that if the molecular concentration of the external medium is lower than that of the cell contents, as in the case of plants and unicellular animals, there must be a constant osmotic pressure tending to draw water into the cell. In plant cells with a cellulose envelope, undue distension of the cell is prevented, but a high internal pressure

results. This is known as "turgor" and is responsible for the rigidity of many upright flower stalks, which are soft and yielding in its absence. In most tissues of the higher animals, the cells are surrounded by a liquid of very nearly the same osmotic pressure as themselves, as can readily be seen in the case of the red blood corpuscles. Thus, the tendency to swell by osmosis is avoided. But in cases such as those of protozoa in water, there is a continuous intake of water which would burst the cell were it not for the periodic rejection by the *contractile vacuole*, as was first pointed out by Marcus Hartog. As this vacuole grows in size, it reaches the cell-membrane and empties itself, the membrane then closing over the orifice. The whole process is repeated periodically.

The existence of a cell-membrane is also of importance in some histological methods. In order that a dye may stain structures within the living cell, it is obvious that the membrane must be permeable to it. In some cases, however, it appears

that the dye may injure the membrane, making it permeable, although the cell is not at once killed. Investigation of the staining properties of the cell during lethal changes would probably afford valuable information, since these reactions seem to be different both from those of the living cell and those of the dead cell. Even in the fixed dead cell, the membrane plays a part according to Benians, who states that Gram-positive and Gram-negative bacteria differ only by the presence or absence of a membrane capable of retaining the compound dye-iodine particles.

The fact of the membrane being an integral part of the cell mechanism makes less puzzling such observations as those of Warburg on the increase of oxidation in egg-cells when exposed to sodium hydroxide. This alkali does not enter the cells, as can be shown by previously staining them with neutral red. Shearer, again, has shown that a similar increase in oxidation occurs in the process of fertilization *before* the sperm cell has actually entered.

Changes in Activity

Owing to the mode of its formation, the cell-membrane cannot be looked upon as a permanent structure. It changes reversibly under the influence of changes in the cell and in equilibrium with them.

Lillie and others have shown in various ways that the membrane becomes permeable in the state of excitation of the cell. Thus at this time substances can be taken into the cell to which it was inaccessible in the state of rest. Osmotic observations on resting cells, for example, show that they are impermeable to glucose and to amino-acids. But these are necessary foods for the protoplasm, and until the discovery of permeability in activity was made, a difficulty was felt. It is of interest to call to mind that glucose is especially needed for the supply of energy in activity and that it is just in this state that it can enter the cell.

Active muscle takes up potassium,

whereas the resting cell does not. After having entered in during activity, it does not escape during subsequent rest under normal conditions. If, however, there is no calcium present, the potassium escapes, owing to the necessity of calcium for the preservation of the normal condition of the membrane (Mitchell, Wilson and Stanton).

CHAPTER VIII

ELECTRICAL PHENOMENA IN LIVING CELLS

IN this chapter we propose to discuss some results which follow from the impermeability of the cell-membrane to certain ions and its permeability to those of opposite charge.

Electrical Conductivity

Since the conductivity of a solution depends on the movement of the ions contained in it to the oppositely charged electrode and their giving up to this of the

charge they possess, it is clear that freedom of access to the electrode is essential. If a sac consisting of a membrane which is impermeable to electrolytes, but permeable to water, a so-called "semi-permeable" membrane, containing a solution of some salt is immersed in a solution of the salt, that portion of the way between electrodes at opposite sides of the vessels which is occupied by the sac does not serve for the purpose of conducting the current. Hence the resistance of the system is greater than it would become if the membrane of the sac became free for the passage of ions, so that the whole space could serve for conducting the current.

The cell-membrane is usually impermeable to the ions present in the cell, so that this cell acts more or less as a non-conductor. If the corpuscles in blood are so treated as to produce hæmolysis, it is clear that their resistance disappears and the conductivity of the blood increases. This is the basis of Stewart's method of determining the relative proportion of corpuscles and

plasma. And also, as it appears to me, of the experiments of Osterhout on the increase of conductivity of vegetable cells under the influence of sodium and its decrease again when calcium is added. In the latter case, the cells are normal as regards their permeability ; in the presence of sodium salts alone the membrane loses its semi-permeability and therefore conducts well. It has also been shown that excited muscle has a lower resistance than when at rest. This fact serves to confirm the view that the excitatory state is accompanied by increased permeability.

It is of interest to recall the fact that in the electrolytic process used by Morse for making his osmotic cells, the progress of the membrane towards perfect semi-permeability was indicated by a steadily increasing electrical resistance, due to its becoming less and less permeable for the ions of the solutions forming it.

Electrical Changes in Excitation

It was pointed out by Ostwald in 1890 that in order that a membrane should be impermeable for a particular salt, it is sufficient that it should be impermeable for one only of the two opposite kinds of ions into which the salt is dissociated. The other cannot escape owing to the electrostatic attraction of its fellow, which however allows it to go far enough to form on the outer side of the membrane a layer of the permeable ions. As Ostwald pointed out, such a process results in the presence of an electric charge on the membrane, or a potential difference between the two sides of the membrane. We have already considered the case of Congo-red, where the indiffusible ion is colloidal, but the facts apply also to substances in true solution, if the membrane is an appropriate one. Measurements in the case of Congo-red indicated to me that when solutions of different concentration were on opposite

sides of the membrane, the values of the potential difference were in agreement with the Nernst formula for a concentration battery. It has been pointed out that a system of this kind forms a diagram of the conditions present in a cell with a metallic electrode. It should be noted that the source of the electromotive force is in the ions of the non-diffusible electrolyte itself and would be present if no other ions existed in the solution. Such then is the basis of the explanation of the potential differences found in living cells. It is necessary to be clear about the matter, because some confusion has arisen in connexion with the part played by the Donnan membrane effect. In the course of the work that I was doing on the osmotic pressure of congo-red, I noticed that foreign salts, such as sodium chloride, although both of its ions were freely diffusible through the membrane, were never equally distributed between the inner and outer solutions. Donnan came across the fact at the same time, and in 1911 published a

thermodynamic treatment of the problem, showing how the relative concentrations could be calculated. Since the potential difference in the limiting case with pure water, as mentioned above, depends on the layer of sodium-ions on the outside of the membrane, it is clear that a different concentration of these ions on the two sides implies a modification of the potential difference of the pure solution. This can be calculated from Donnan's equations. But of course it cannot properly be called the origin of the potential difference. There would be none in the absence of the indiffusible ions and it would be maximal in the absence of the diffusible salt with which the Donnan theory is concerned. Owing to the presence in certain cases of diffusible salts, Donnan's theory of membrane equilibrium is of much importance in some physiological processes.

Suppose next that the living cell-membrane is permeable to anions, impermeable to cations, as we find by experiment to be the case with the red blood corpuscles.

It is easy to see that it will have a negative charge on the outer surface, conferred by the layer of anions. The cells of secreting glands appear to behave in the same way. Whereas the muscle cell is permeable to cations, impermeable to anions, and has therefore a positive charge, as can be seen by injury to one part of it.

An interesting case of surface charge which can be treated in a similar way is the system described by Beutner, where two immiscible liquids, a solution of toluidine thiocyanate in toluidine, and one of potassium thiocyanate in water, are in contact. The potassium-ion is insoluble in toluidine, whereas the thiocyanate one is. The latter wanders out as far as the attraction of the potassium-ion permits it and thus forms a Helmholtz double layer at the interface.

Another source of potential difference should be mentioned, although it is doubtful if it has much physiological importance. If a solution of an electrolyte comes into contact with water, the faster moving ion

diffuses out to a slight degree in advance of its fellow, and thus gives rise to a potential difference. This however is not permanent, unlike the membrane effect in cells.

The effect of an increased concentration of carbon dioxide in the blood in giving rise to an increase in the "alkali reserve" of the plasma, as investigated particularly by Hamburger, has been recently shown by Warburg in Copenhagen to be satisfactorily explained by an interchange of anions (Cl' and HCO_3') through the membrane, as regulated by the Donnan membrane effect. The swelling of the corpuscle is explained by the resultant increase in osmotic pressure within it. The net result is an increase in the relative proportion of HCO_3 ions to chlorine ions in the plasma, the cations remaining as before.

Another interesting fact relating to the permeability of the cells is that carbon dioxide appears to be more powerful as a stimulus than mineral acids of equal

hydrogen-ion concentration. These latter do not readily pass into the cell, because the membrane is impermeable to the hydrogen-ion as to other cations. At least I find evidence that the statement applies to blood corpuscles. Now Jacobs has pointed out that carbon dioxide dissolved in water (not as the ions of H_2CO_3) must be readily diffusible through the cell-membrane, because it is continually being produced and must be removed. When it has entered the cell from outside, it combines with water to form the ionized acid in immediate contact with sensitive cell structures. This seems a very reasonable view and explains some rather puzzling phenomena.

Similar considerations apply to solutions of ammonia. In an acid solution of ammonium chloride, the contents of a living cell may become alkaline, owing to the permeability of its membrane for undissociated NH_3 or NH_4OH . Thus carbonic acid and ammonia as alkali possess special properties in relation to changes of reaction within the cell.

We pass on now to what happens to the electric state of the cell when activity is entered into. Such electric changes have been known for a long time in muscle and nerve and various theories have been put forward to account for them. Leaving on one side the older views of Du Bois Reymond and of Hermann, which were propounded before the phenomena of electrolytic dissociation were known, we may discuss what seems to be the most in accordance with the facts and is essentially that put forward by Bernstein. This investigator realized that the phenomena must be accounted for by the movements and changes in local concentration of ions. Evidence for this was afforded by the temperature coefficient of the potential difference of injured muscle, which was too low to be that of a chemical reaction. Loeb and Beutner also found that the value of this potential difference depended on the ions present in the electrodes used.

Starting from the condition of the muscle

or nerve cell as described above and represented in diagram in Figure 4, we note that if any two points on the normal cell are led off they will be equi-potential, because the inner member of the double layer is inaccessible. But if the membrane

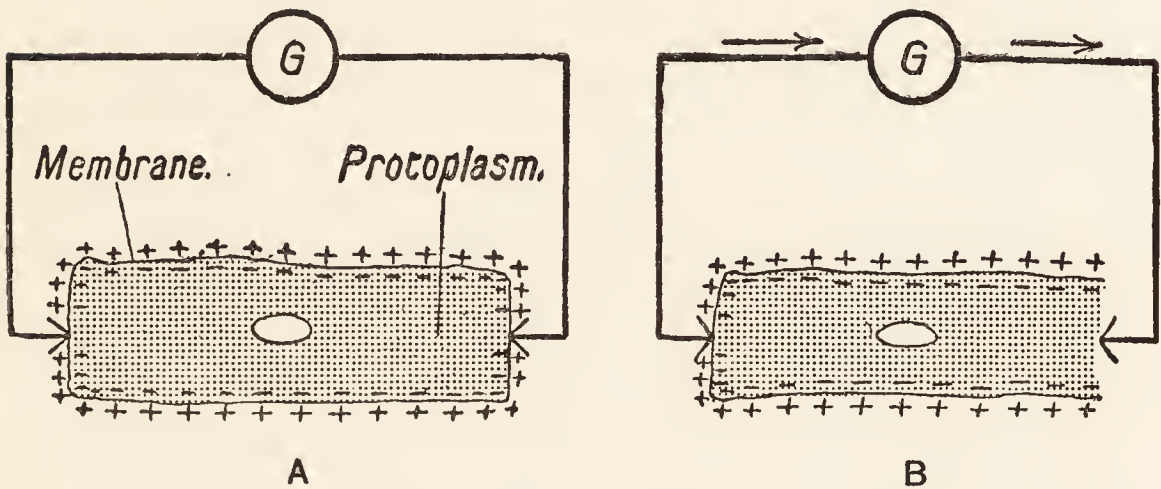


FIG. 4. Diagrams of the Electrical State of the Resting Cell (A) and of that of the Active Cell (B).

is injured or made permeable at any spot, the result is equivalent to its disappearance; the ions which were separated by the membrane (which is sometimes said to be "polarized") mix together, the potential difference disappears and the electrode here is enabled to lead off, through the

contents of the cell, the internal member of the double layer, which is negative to the outside. Thus the "negativity" of an injured or excited spot should rather be spoken of as positivity of the normal spot. The current so produced is the "demarcation current," which naturally disappears as the electrolytes contained in the cell diffuse away through the injured part.

Suppose next that the cell showing this demarcation current is stimulated so as to become permeable at the other electrode also. The potential difference is now absent at both electrodes and there results a diminution or abolition of the demarcation current: this diminution was originally called "negative variation." If both electrodes are on normal spots and the excitatory state passes as a wave under each in turn, it is clear that the effect is a "negativity" of the electrode first met, followed by that of the other, the so-called "diphasic variation."

We have already considered the evidence

that excitation means increased permeability.

The electrical effects in secreting glands are rather more complex, because of the two kinds of innervation here present, "secretory" and "trophic," typified by the chorda tympani and the sympathetic

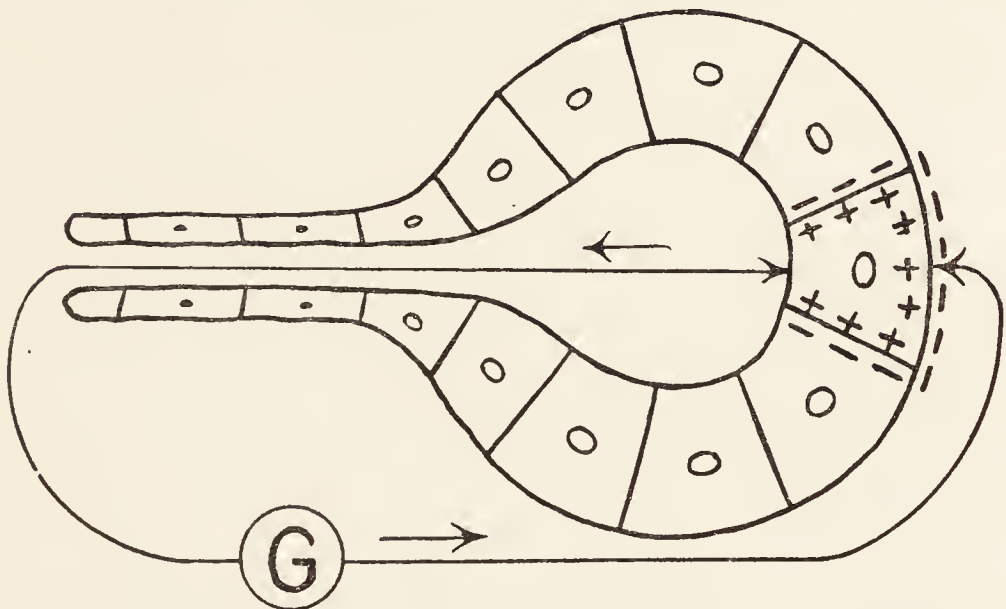


FIG. 5. Diagram of Electrical State of Gland Alveolus.

in the dog. The result of stimulating the former is in some way connected with the flow of water through the cell as a result of increased permeability of that part of it which is in relation with the lumen of the duct, as we shall see later. The diagram of Figure 5 will assist in making

clear the electrical result of this process. The direction of the excitatory change is in the opposite direction to that of muscle. Anrep and Daly have recently demonstrated a similar effect in the pancreas excited by secretin (Figure 6).

The effect of stimulating the sympathetic supply to the sub-maxillary gland in the dog is very small and in the opposite direction to that of the chorda. It seems to be connected in some way with the solid contents of the secretion.

It may be mentioned that scarcely any advance has been made in our knowledge of the electrical changes in glands since the work of Bradford and the present writer in 1885. It is now time for a renewed attack from the point of view of modern knowledge of electrical phenomena.

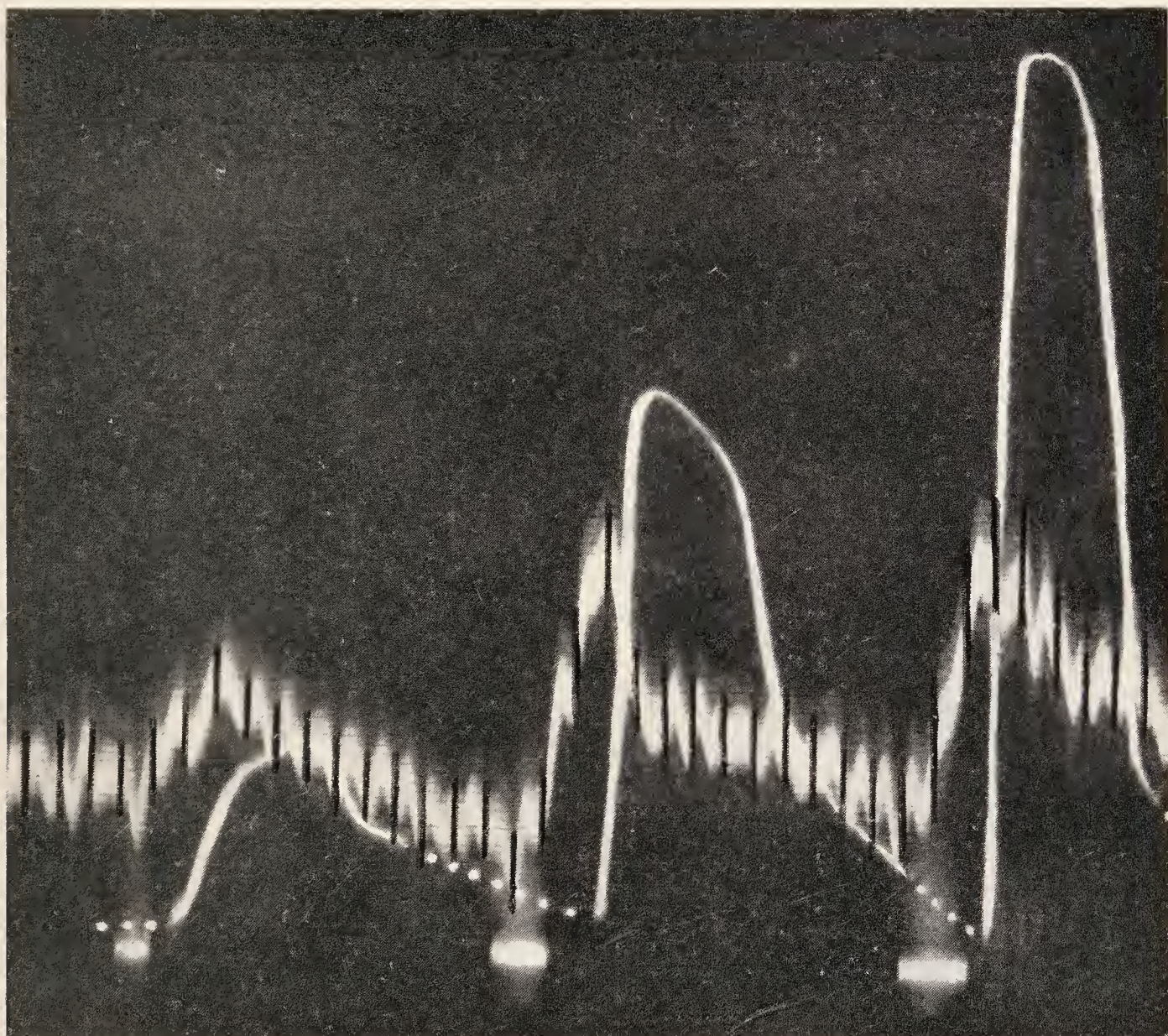


FIG. 6. Electrical Change in Pancreas (Anrep and Daly)

Three injections of secretin, 2, 4 and 8 c.c. as marked below. Broken curve—galvanometer deflection. Plain curve—flow of secretion. Each interruption on the electrical curve corresponds to 25 secs. The small waves on the electrical curve are due to intestinal movements.

CHAPTER IX

PROTOPLASM

FROM consideration of the cell-membrane we may turn naturally to that of the contents of the cell. Protoplasm in its simplest form, as in the pseudopodia of the amœba appears to be of a liquid nature. Chambers found that it opposed no appreciable resistance to the drawing of a needle through it, and the needle left no track behind. But even this apparently clear and structureless substance is in reality colloidal in nature, as I was able to show by intense dark ground illumination, when we see innumerable minute shining particles in Brownian movements. The presence of this movement confirms the view of the liquid nature of the system, because otherwise the particles would not be free to move.

Viscosity and Gelation

On electrical stimulation, I noticed that the Brownian movement above mentioned ceased suddenly for a brief period. Hence the liquid state of the protoplasm had changed to one similar to that of a jelly. Seifriz, Chambers and others observed increased viscosity in the protoplasm of cells in activity, in fertilization, mitosis, etc. In plant cells, this can be detected by an increase in the time taken for grains to fall through the cell when inverted in position. The changes referred to seem to be due to a removal of water by the dispersed phase, or other structure, and the resulting increased concentration of the system as a whole. This phenomenon has already been mentioned in connexion with the synthetic action of intra-cellular enzymes.

If we may look upon this increased viscosity or gelation as a sign of activity, it is of interest to call attention to the fact

that the protoplasm of pseudopodia is liquid. This suggests that these protrusions are not produced by cell activity but by surface changes due to arrival of substances in the outer medium which lower surface tension. The question is still under discussion.

Fixation in Histology

The various fixing agents produce coagulation structures of manifold forms in the colloidal system of protoplasm, such as networks, fibrillæ, granules and so on. Hardy has given a detailed description of these, but his work has been strangely neglected by histologists, who frequently fail to realize how many of the structures they describe are not present while the cell is alive. Much more work is needed on the living cell, and I would especially state my appreciation of that done by Chambers in micro-dissection and manipulation and of that of the Lewis' on tissue culture.

It would seem that the appearance of

networks in living protoplasm as described by some observers is due in the main to faulty methods of illumination, leading to diffraction images. It is easy to obtain what look like definite structures from a mere accumulation of dots. Mott and Marinesco, independently, made examination of living nerve cells under dark ground illumination and came to the conclusion that both Nissl granules and neuro-fibrils are the products of fixing agents. The contents of the living cell which become coagulated into these structures are present as minute particles with a vigorous Brownian movement. Hence the protoplasm here as elsewhere is a liquid colloidal solution.

We have seen above that the production of pseudopodia is in intimate relation to local changes of surface tension. It is clear that the greater tension over the main body of a cell would cause protrusion of a part where it is less in magnitude. Whether there may be, in addition to this, something more like muscular contraction

in a layer of visible thickness is not yet certain. When a cell is in contact with a solid surface, the forces of surface tension at the different interfaces must be considered. The usual result is to tend to draw out the protoplasm in a thin layer. The adhesion of protoplasm to a surface wetted by it is also an important factor. These various phenomena have been investigated in detail by Tait.

The clotting of blood by the agency of surface forces where it is in contact with material wetted by it, and the retardation of the process by coating canulæ and so on with a "greasy" layer of paraffin or vaseline are familiar facts.

The Rôle of Structure

But, as it is scarcely necessary to remark, the protoplasm of the cell as a whole is a much more complex system than that of the clear pseudopodia. It is really an extraordinarily interdependent heteroge-

neous system of many phases, solid and liquid, separated by membranes, of whose intimate arrangement very little is known. Nevertheless, there are numerous experimental results that show how important this structure is, as it exists in the living state.

In general, it is by its agency that the chemical energy of food is changed to other forms of energy, mechanical, electrical and so on. It may be noted that it is this process of conversion of energy that constitutes what we call vital phenomena, and one of the characteristics of living organizations is that the mechanism is such as to avoid as far as possible the loss involved in the intermediate passage through heat. There is a direct conversion of chemical or radiant energy into other useful forms. This is not, of course, peculiar to the living body, and is not to be looked upon as in any sense a reversal of the second law of thermodynamics, as seems to be suggested by some writers. There is no increase in free energy, merely less loss than might have occurred.

The part played by the structure of the cell may be pictured somewhat as follows. If we imagine that a petrol motor is broken to pieces, the parts mixed with the combustible fuel and then ignited, nothing but heat is obtained. Whereas if the fuel is burned in relation with certain parts of the machine as a connected whole, a large part of the chemical energy is converted into useful mechanical work.

In the cell, also, the most striking facts that have come to light concern oxidation processes. Rubbing up muscle tissue with sand destroys its power of oxidizing lactic acid (Fletcher and Hopkins, Harden). A similar procedure greatly reduces the fermentative power of yeast cells (Warburg, Meyerhof).

The structure here in question is more subtle than that shown by the microscope. If the cell is killed by the action of acetone, the microscopic structure is well preserved, whereas the consumption of oxygen is reduced to one-third. That it is not entirely stopped is in itself a

point of interest, but of course this residual process comes to an end after a time.

Otto Warburg regards surface condensation on structural units (adsorption) as the main factor, but it is probable that ultra-microscopic reaction chambers, bounded by reversible semi-permeable membranes, also play a part. This investigator makes the interesting suggestion that the consumption of energy by cells apparently inert is required to maintain "structure" against diffusion gradients, potential differences, loss of semi-permeability and so on. Something of the same kind is doubtless concerned in the comparatively large consumption of oxygen in mitosis and in the multiplication of cell substance.

Effects of Freezing

If a solution is slowly cooled down to the temperature at which it begins to freeze, it is well known that the ice which separates is pure solidified water. The

solutes, colloidal or otherwise, are thus left in a more concentrated form. If, however, it be cooled rapidly to a much lower temperature, namely, that at which the saturated solution freezes, the whole solidifies together of a uniform composition. There is no separation of pure ice. This temperature at and below which the solvents and solutes freeze together may be called the "eutectic" temperature, as it is essentially the same phenomenon as that known by this name in the case of metallic alloys.

The process applied to living cells has shown that if the eutectic temperature is reached sufficiently quickly, no disorganization of the cell occurs. On thawing, it resumes its normal activity. If, on the contrary, pure ice is allowed to separate, owing to slow cooling, the cell structure is destroyed and no recovery is possible. The practical difficulty is to ensure a sufficiently rapid cooling of the interior cells in an organism of other than very small size. Kühne froze the staminal hair cells of *Tradescantia* at -14° C. and found

protoplasmic movement to return on thawing. Miss Foster succeeded in retaining electrical excitability in frog's muscle and Rahm has frozen rotifers in liquid helium with ultimate recovery.

Altmann has applied the process to the desiccation of tissues in preparation for the cutting of sections. I have myself confirmed the possibility of doing this, but the difficulty arises in the subsequent treatment of the sections to remove the paraffin in which they were imbedded. They fly to pieces in any watery medium. It is probable that they must be gradually brought back to their normal content in water by exposing them to water vapour at a low temperature and slowly bringing up to room temperature. This seems worth trial.

Another application of the facts is a commercial one, namely, the brine-freezing process of preserving fish and meat. The frozen material regains its normal structure on thawing, so that the contents of the cells do not ooze out and in other respects

the preservation is more perfect than in the ordinary slow freezing.

The freezing point of protoplasm, that is, the temperature at which ice begins to separate on slow cooling, is about -2° C. Nevertheless it is found that a particular mould ("black spot") grows on meat in cold storage at -7° C. It is clear that the protoplasm cannot be in a frozen state, and the explanation is probably that it is contained in narrow capillary tubes of mycelium and that surface forces of cohesion by their pressure effect lower the freezing point to a sufficient degree.

Tissue Culture in Vitro

Work of much importance is in progress at the present time and in various places based on Ross Harrison's discovery that tissues can be cultivated in glass cells under the microscope. The use of the animal's own plasma has been found to be unnecessary, and the Lewis' have even

succeeded in growing tissues in Ringer's solution. To avoid vacuolation of the new cells, it was found that glucose is necessary, even in the presence of protein.

The cells in the centre of the transplanted bit of tissue usually degenerate and die, probably from deficiency of oxygen, and their constituents presumably serve as food for the new outgrowth. When the tissue is a pure one, unmixed with cells of a different nature, the new cells tend more and more to become of an embryonic type, a process called by Champy "de-differentiation."

Influence of One Tissue on Another

This de-differentiation does not occur if cells of another kind are present. Thus, the co-existence of connective tissue enables renal tubules to grow out normally ; retinal cells require the presence of pigment cells, etc.

There is evidence that the effect is only exerted in immediate proximity to the

cell from which it proceeds. If a cell wanders away from the parent mass, it loses its differentiated structure or the cells it produces are simple embryonic ones. If this is so, it is difficult to attribute the effect to specific chemical products. At present it is only possible to speak of it as an "influence" of some kind.

Indeed, specific or individual properties remain for the most part unexplained as yet, although Dudley has made some advance in the study of proteins by the method of "recemization." In this case, there is great variety in the possible pattern or order of arrangement of the amino-acid constituents.

CHAPTER X

MUSCLE, NERVE AND GLAND

Muscular Contraction

THE work of A. V. Hill has shown that the energy set free in a muscular contraction is proportional to the length of the muscle fibres at the time of their beginning activity. The longer a particular muscle is, the greater is the energy produced. Stretching a muscle does not increase its volume, but it increases the length and with that the area of surface of some structures arranged lengthwise. The source of the energy is therefore from surface forces rather than from osmotic forces. Thus, the surface tension theory of Bernstein is favoured. This view is also supported by the fact

that the development of energy has a negative temperature coefficient, like other phenomena dependent on surface tension. The energy of a contraction is less at 30° C. than at 15° C.

In a classical paper published in 1909, Haber and Klemensiewicz put forward the view that such a surface tension change might be produced by adsorption of hydrogen-ions on the fibrillæ of the muscle cells. These ions, of course, arise from the lactic acid, known to be formed.

I may remark, incidentally, that the title of my lectures is a translation of Haber and Klemensiewicz, "Phasengrenzkräfte."

If it is the hydrogen-ion that is the necessary agent in the contraction of muscle, a certain difficulty is removed with regard to the supposed consumption of fat by muscle in place of carbohydrate. This remains a disputed question. It is perhaps of interest, however, to note the presence of hydroxybutyric acid in diabetes, which might conceivably replace lactic acid, so far as the formation of hydrogen-ions is concerned.

In diabetes, hydroxy-butyrlic acid is formed in the defective oxidation of fat and might occur in muscle. At the same time, it is suggestive that even when no carbohydrate is taken in the food, the body makes glucose from amino-acids, and this may be essential for the muscular process. Determinations of the lactic acid content of muscles in diabetes would be of interest.

In accordance with the considerations brought forward in the preceding chapter, we find that the efficiency of the muscular machine in the engineer's sense is too great to be that of any possible heat engine at temperatures compatible with life. Thus chemical energy must be converted into mechanical work without passing through heat.

The "Law of the Heart"

Starling has introduced this name to express the application of the dependence of work done on initial length to the phenomena of the output of the heart.

It is found that all these follow from the results of changes in diastolic filling of the ventricles, that is, from the varying lengths of the muscle fibres at the moment of systole. It is naturally a difficult matter to distinguish the effects due to tension from those due to length, but in certain experiments by Patterson and Starling, these two factors were found independent and the output was a function of the length, not of the tension.

Tonus and Posture

It is clear that if it is the presence of the hydrogen ions on the fibrils that cause contraction, this state will last until the lactic acid is removed in some way, a process that may be expected to require the supply of energy. A. V. Hill has recently found, indeed, by the aid of exact analysis of the time course of the production of heat, that there is a secondary rise occurring in the relaxation period, but

preceding that associated with the oxidation process which restores the original potential energy to the system.

The existence of this special process, whose object it is to remove the acid, tempts me to recall the suggestion that I made in another place, namely, that the remarkable phenomenon of tonus, in which there is no consumption of energy, or only a minimal one, might be due to the absence of or the failure to put into action this mechanism for removing acid. There are various facts which support this view, but further investigation is needed and discussion would be premature.

The view of Botazzi, that tonus is due to contraction of sarcoplasm, does not seem to me to be satisfactory. But it is not a simple problem for experimental test.

The Nerve Synapse

It is to be expected that in common with other cells of the organism those of

the nervous system, the neurones, should possess a semi-permeable membrane. Where two neurones come into contact with one another, or rather where the ramifications of the axon of one come into contact with the dendrites of the other, there will be a semi-permeable membrane. Sherrington has given the name of "synaptic membrane" to this structure and has called attention to the opportunities it gives for the interchange of ions in a definite manner, for the establishment of electrical potentials and osmotic phenomena of various kinds.

It doubtless plays a part in the transmission of the excitation process from one neurone to another. The possibility of the membrane becoming permeable in excitation, as previously described, suggests in a general way the method by which the electrical disturbance in a nerve fibre may set free energy in the cell body, or centron, of another neurone, by which the impulse is enabled to pass on into particular channels.

Excitation and Inhibition

Since these two processes are opposite in nature, they differ essentially merely in the fact that whatever phenomenon takes place in the one, it may be assumed that the contrary one takes place in the other.

In the process of reciprocal innervation in reflexes, for example, it appears that one and the same afferent nerve fibre branches and terminates in two different ways on the motor centres of antagonistic groups of muscles, flexors and extensors, for instance. The identical nerve impulse thus excites one set of neurones, inhibits the activity of the other set, owing to the different manner of terminating, or to be more precise, owing to the different nature of the synaptic membrane in the two cases.

Although it may be at the present time too hypothetical to say more, it is tempting, in view of the two kinds of polarized permeability, about which we have spoken

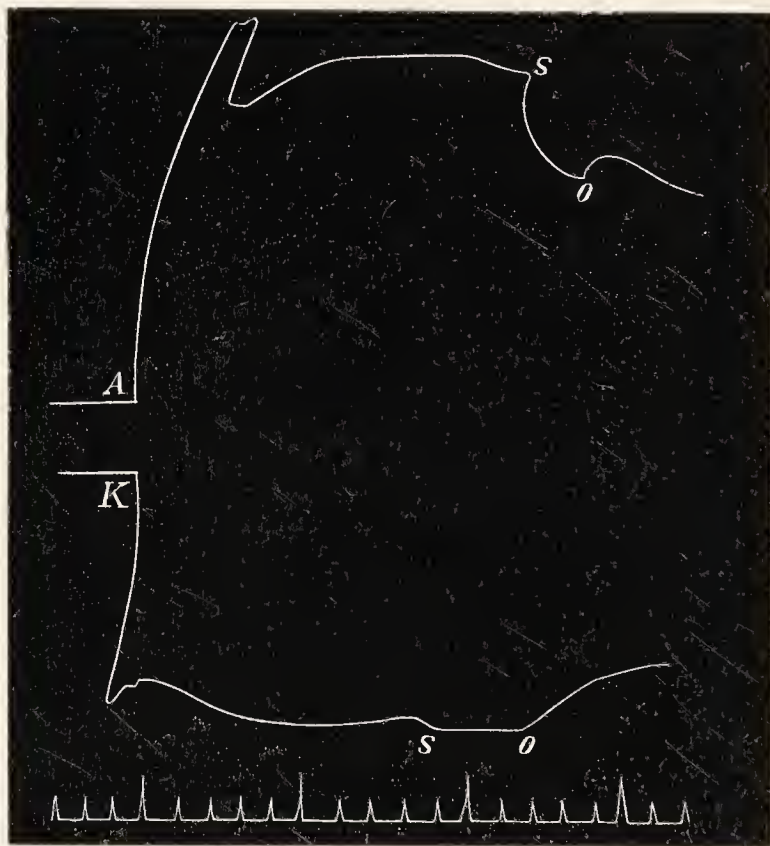


FIG. 7. Inhibitory Action of Anode (Biedermann)

Sartorius muscle fixed in the middle, so that each half contracts in opposite direction. At *A K*, muscle stimulated after action of veratrin. At *s*, application of galvanic current, anode on that half giving upper tracing. *o*, break of current.

above, namely, one for anions only, the other for cations only, to suppose that one of these is inhibitory, the other excitatory. The opposite sign of charge, together with the electrical nature of the nerve impulse and the fact that the action of the anode of a constant current applied to nerve or muscle is inhibitory (Fig. 7), while that of the cathode is excitatory, lend some support to such a view.

Secretion in General

In our previous discussion of the electrical changes in secreting glands, we saw that an explanation of the potential difference associated with the flow of water is possible on the lines of an increased permeability of that part of the cell in relation with the lumen of the alveolus. It may be profitable to devote a few lines to the application of the theory to the secretory process as a whole. Space does not permit a complete discussion of the facts which afford evidence

for the following statements, but it may be understood that it exists.

While a gland is at rest after a preceding period of activity, there takes place an accumulation of some precursor of the specific constituents of the secretion. This appears to be a complex system of large molecular dimensions, and to produce it in the cells a supply of energy is required. This energy is obtained from the oxidation of glucose. In certain glands, we see this precursor, or one of them, in the form of visible "zymogen" granules. The process described proceeds without the necessity of nervous stimulation and has the character of a balanced chemical reaction proceeding to equilibrium automatically.

When the gland is set into activity by stimulation of the nerve supplying it, the precursor in question is partly split up into smaller molecules. Thus the osmotic pressure in the cell rises, it attracts water from the lymph, while at the same time the duct end of the cell becomes permeable. Thus the water escapes, carrying with it

cell contents. The process continues as long as the osmotic pressure of the cell contents is higher than that of the lymph.

Some idea of the mechanism at work may be obtained by considering a vertical tube, open at the top, closed with a semi-permeable membrane at the bottom, filled with sugar solution and standing with its lower end immersed in water. Water enters by osmosis at the lower end and a solution of sugar escapes at the top to make room for the water. A completely permeable membrane may be tied over the top and the tube completely immersed in water. If a solution of Congo-red be used in place of sugar, the dye can be seen to escape from the permeable end ; and by renewal of the outer water the whole of the dye can be washed out in process of time.

In the case of the secreting cell, it appears that the actual process of secretion is not itself accompanied by consumption of oxygen. Thus, in its main features, it is strikingly similar to the mechanism of the muscle system.

Formation of Lymph and of Urine

It is very doubtful whether any true secretory process occurs in these cases. The first stage in the formation of urine is shown by overwhelming evidence to be a process of filtration, or ultra-filtration, by the membrane of the blood-vessels forming the glomeruli of the kidney. Like the walls of the capillaries in other parts of the body, this membrane is, as regards permeability, similar to parchment-paper or collodion; that is, permeable to substances in true solution, impermeable to colloids. This being so, any osmotic pressure possessed by the colloids of the blood plasma must show itself by attracting water and, unless opposed by a greater filtration pressure, no liquid will pass outwards. Starling showed that these colloids have an osmotic pressure, as measured against the filtrate from which they were removed, of 35 mm. to 40 mm. of mercury. Hence, unless the arterial pressure in the glomerulu,

which is that in the small arterioles of the general circulation, exceeds this value, there will be no urine from the kidney and no lymph in other parts of the body. This is in accordance with fact. It will be noted that the energy necessary to do the work against osmotic forces is afforded by the blood pressure produced in the arteries by the contracting muscle of the heart.

The further fate of the filtrate in the two cases differs. Let us consider that of the lymph first. As the blood pursues its course from the small arterioles through the capillary network, the pressure in the vessels soon falls below that necessary for filtration and further on becomes lower than the osmotic pressure of the blood colloids. At this situation, the liquid previously filtered through is therefore more or less reabsorbed. What is left unabsorbed passes away in the lymphatic channels to be returned to the blood via the thoracic duct.

Next, suppose that the blood is diluted by the addition of a solution which contains

no colloids with an osmotic pressure, as by an intravenous injection of saline. It is clear that the concentration, and therefore the osmotic pressure, of the blood colloids is reduced. The pressure available for filtration, that is, the difference between the osmotic pressure of the blood colloids and the arterial pressure, is increased. The rate of filtration rises, and in the case of the capillary circulation, the length of the path traversed before reabsorption begins increases. Hence the liquid introduced leaves the circulation rapidly, actually in twenty to thirty minutes. Thus, if we want to produce a permanent increase in the volume of the blood circulating, as after hæmorrhage or in wound-shock, we must add a colloid with an osmotic pressure equal to that of the colloids in the blood. In the so-called "gum-saline," which was introduced by me during the late war, this is done by the addition of 6 or 7 per cent. gum acacia to 0.9 per cent. saline.

It will further be evident that if the capillaries lose their impermeability to colloids,

as happens in late stages of wound-shock, the normal mechanism is put out of action, so that there is no longer any force resisting filtration of plasma, including its colloids. The blood becomes concentrated as regards corpuscles, while any fluid introduced, blood or gum-saline, escapes. There is evidence, however, that the normal state of the capillaries can be restored by repeated small injections, provided that the morbid state is not too severe or has not lasted too long.

It will be realized that in the process of filtration from plasma as described above, all the diffusible constituents, those in true solution, pass out with the water. Now these are of two kinds, the valuable material, such as amino-acids and glucose for food and certain necessary salts, such as sodium chloride, on the one hand; the waste products, typified by urea, on the other. The escape of useful materials does not matter in the case of the lymph, since they are returned to the circulation. It is, moreover, necessary that the blood

vessels should be permeable to them in order that they may reach the body cell. But they must not be lost to the organism in the urine to any greater extent than can be avoided. We find, accordingly, in the renal tubules, a mechanism of an opposite direction to that of secretion, by which water containing the valuable materials is reabsorbed while the excretory products are allowed to pass away. Further details would be out of place here, but it may be noted that it is held by some that certain cells of the tubules have the power of excretion as regards urea, uric acid, etc. The evidence for this is not strong, and the simpler theory, as propounded by Cushny, is capable of accounting for all the facts.

The Action of Drugs

The possibility that at all events some substances exert their physiological or pharmacological effects by action on the cell-membrane without entering the cell itself

must be reckoned with. Straub has brought evidence of this kind as regards calcium and strophanthin. The case of sodium hydroxide has been mentioned above.

The remarkable effect of strychnine in so changing the synaptic membrane of neurones that an inhibitory process is converted into an excitatory one, as discovered by Sherrington, and the converse effect of chloroform in converting excitation into inhibition, described by the author, may be referred to here. One may hazard the suggestion that changes in the sign of the ionic permeability of the membrane may be concerned. But on the other hand, there is the alternative view that the results are due to the opening or closing of collateral paths by effects on the permeability of particular neurones. The problem is not yet solved.

Matthews regards such theories of adsorption of drugs as being a step backwards. But Dale's work has shown that chemical combination alone is inadequate to explain all the facts.

CHAPTER XI

GENERAL REMARKS

CERTAIN general conclusions may be drawn from the facts to which attention has been directed in these lectures. The processes of muscular contraction and of secretion especially serve to show how complex is the intermingling of chemical and physical phenomena in physiology. It is indeed difficult or impossible to find a scientific justification for the separation of biochemistry from biophysics. Such specialization runs great risk of a one-sided and incomplete view of many vital phenomena.

It is true that for purposes of laboratory convenience and owing to the different kinds of apparatus required, a biochemical department is desirable in a complete institute of

physiology. Indeed, even if certain kinds of investigation can be adequately carried out in an independent biochemical institute, it is none the less true that no physiological laboratory can avoid the necessity of its own chemical department. In a similar way, appliances for biophysical work are often required in a biochemical institute. If the departments are spatially separate, a close co-operation must be provided for, and the best arrangement is undoubtedly where they form parts of a common physiological unit.

A biochemist indeed must be something more than an organic chemist occupied with the properties of compounds which happen to be provided for him by the living organism. A good case can be made out for the separation of biochemistry from pure organic chemistry, contrary to what is the case with physiology.

The formation of separate departments of biochemistry appears to have been greatly due to the need for the cultivation of aspects of physiology which were unpro-

vided for in the laboratories attached to medical schools, where it was natural to overlook problems not obviously connected with human physiology. But what is really wanted is the establishment of more laboratories and chairs of pure physiology, including both animals and plants—of general physiology in its widest aspect. It was always insisted on by Claude Bernard that physiology is an independent science, to be cultivated for its own sake and irrespective of ultimate practical application. And even as regards these, it must not be forgotten that they include many things in addition to providing the essential foundation for the cure of disease. The health officer requires more knowledge of the healthy state, that is, of physiology, not so much of the details of the diseased state. We must not forget, besides, the needs of agriculture and indeed of all industries occupied with living organisms.

I also venture to hope that I have made clear the importance of the knowledge of fundamental principles, even to the student

of medicine. Given these principles, it will be found that much of the detail, often learned with great labour, follows naturally. The same statement applies to the practical clinical applications. As it appears to me, the movement in some quarters to confine the physiology taught to the medical student to that part of the science which is at the moment seen to be of immediate direct application to clinical practice is to be regretted. Apart from the intellectual loss involved, it is never possible to say what may not prove to be of vital importance at no distant date. Before the introduction of electro-cardiographic methods, the phenomena of the electrical changes in muscle and nerve were looked upon as very "academic" physiology. It must not be overlooked, moreover, that the essentials of this knowledge were obtained from experiments on the frog, which the student seems inclined to despise. If time is too limited to master fundamental laws as well as special processes, it would be better to omit the latter. Opportunities should be

present during the clinical course for more detailed attention to these, and the movement for continuation of physiological instruction at this period is to be welcomed.

It is to be regretted that, at all events in England, the want of sufficient preliminary training in general science puts a difficulty in the way of the physiological teacher, who has to devote time which should be available for his proper work to necessary chemical and physical doctrine which ought to have been acquired previously.

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